Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supporting Information

A Thiol-ene Mediated Approach for Peptide Bioconjugation Using 'Green' Solvents under Continuous Flow.

Inés Rabadán González,[a] Joshua T. McLean,[a] Nikita Ostrovitsa,[a] Sheila Fitzgerald,[b] Andrea Mezzetta,[c] Lorenzo Guazzelli,[c] Donal F. O'Shea,[b] and Eoin M. Scanlan*[a]

a. Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse Street, Dublin 2, Ireland.
b. Royal College of Surgeons in Ireland, RCSI, York House, York Street, Dublin 2, Ireland.
c. University of Pisa, Via Bonanno 33, 56126, Pisa, Italy.

E-mail: scanlae@tcd.ie

Table of Contents

General Experimental Details

All commercial chemicals used were supplied by Sigma Aldrich (Merck), Fluorochem, VWR, Carbosynth and Tokyo Chemical Industry and used without further purification unless otherwise stated. Deuterated solvents for NMR were purchased from Sigma Aldrich (Merck) or VWR. Solvents for synthesis purposes were used at GPR grade. Anhydrous CH_2Cl_2, THF, CH_3CN and Et_2O were obtained from a PureSolv MD-4EN Solvent Purification System. All UV reactions were carried out in a Luzchem photoreactor, LZC-EDU (110 V/ 60 Hz) containing 12 UVA lamps centred at 352 nm. Silica gel 60 (Merck, 230-400 mesh) was used for silica gel flash chromatography and all compounds were subject to purification using silica gel, unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out with silica gel 60 (fluorescence indicator F254; Merck) and reverse phase TLC silica gel C_{18} 60 RP-18 (fluorescence indicator F254; Merck) and visualised by UV irradiation or molybdenum staining [ammonium molybdate (5.0 g) and concentrated H_2SO4 (5.3 mL) in 100 mL H_2O]. NMR spectra were recorded using Bruker DPX 400 (400.13 MHz for ^1H NMR and 100.61 MHz for ^{13}C NMR), Bruker AV 600 (600.13 MHz for ^1H NMR and 150.90 MHz for ^{13}C NMR), Bruker AV 400 (400.13 MHz for 1H NMR and 100.61 MHz for ^{13}C NMR) or Agilent MR400 (400.13 MHz for ^1H NMR and 100.61 MHz for ^{13}C NMR) instruments. Chemical shifts, δ, are in ppm and referenced to the internal solvent signals (D_2O at 4.79 ppm and DMSO at 2.50 ppm). Coupling constant *J* is measured in Hz. NMR data was processed using MestReNova software. The assignment of the signals was confirmed by 2D spectra (COSY, HMBC, HSQC). Melting points are uncorrected and were measured with a Stuart SP-10 melting point apparatus. ESI mass spectra were acquired in positive and negative modes as required, using a Micromass TOF mass spectrometer, interfaced to a Waters 2690 HPLC or a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC. APCI experiments were carried out on a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC or direct insertion probe in positive or negative modes. Infrared (IR) spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. Reverse phase HPLC was performed on a Shimadzu Prominence system. For analytical HPLC, Ascentis Express 90 Å C_{18}, 100 x 4.6 mm, 5 µm LC column was used. For semi-preparative HPLC a Ascentis C_{18} 5 µm, 110 Å, 250 x 10 mm LC column was used with a flow rate of 4 mL min^{-1}. UV absorption signals were detected with a Photo Diode Array (PDA) detector at wavelengths of 220 nm.

Continuous Flow Set Up for Initial Optimisation of Radical Mediated Thiol-ene Reactions of Glutathione

The flow reactor used was from Syrris Ltd and consists of two Cavro-type syringe pumps with flow rates ranging from 2.5 to 2500 µL min^{-1}. Reagents were loaded into the reactor chip from two pressurized containers A and B. Glass chip reactors of 1000 µL or 250 µL volume with inner S5 channel diameters of 0.25 mm were used and the temperature was controlled using a cooling/heating plate. For photoinitiation a 36 W Mylee lamp covered was placed over the chip assembly along with a box to prevent radiation leakage. Modules of the system were connected with 0.5 mm internal diameter PTFE tubing. The whole system was computer controlled and pressurized with dry N_2 with a back pressure regulator (1-7 bar).

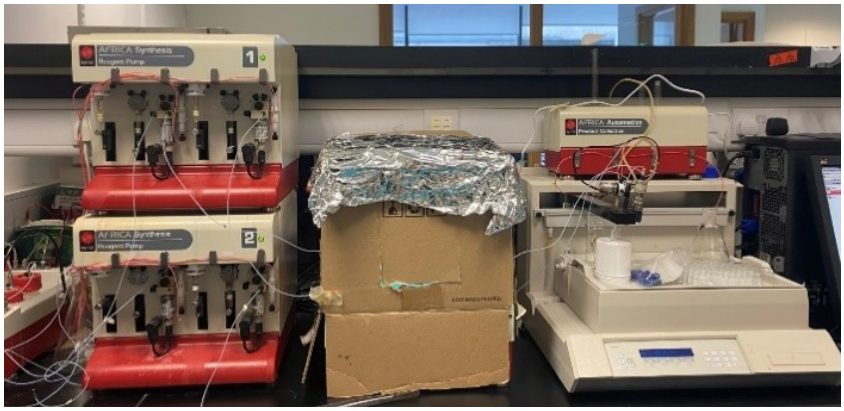


Figure S1: Integrated Syrris Ltd. continuous flow system located in O'Shea Laboratory (Royal College of Surgeons in Ireland, RCSI, York House, York Street, Dublin 2, Ireland) on which initial radical mediated thiol-ene chemistry optimisation was carried out.

Continuous Flow Set Up for Investigation the Radical-Mediated Thiol-ene Reactions in THF/H_2O, Bio-based solvents, DESs and H_2O

The flow reactor consisted of a 10 mL syringe pump which was set up with a flow rate of 0.08 mL min^{-1}. Reagents were loaded into dual syringes at atmospheric pressure. FEP tubing with an inner diameter of 0.8 mm was coiled around a glass insert. This insert was placed inside a Luzchem photoreactor, LZC-EDU (110 V/60 Hz) containing 10 UVA lamps centred at 352 nm. The terminus of the tubing was inserted into a glass vial for sample collection and analysis.

Dual syringe pump, FEP coil in UV oven.

Sample collection.

Figure S2: Continuous flow system within a 110 V UV oven, located in Scanlan Laboratory (Trinity Biomedical Sciences Institute, Trinity College Dublin, 152-160 Pearse Street, Dublin 2, Ireland). Investigation of the radical mediated thiol-ene chemistry of unprotected alkenes in THF/H_2O, DESs and bio-based solvents and H_2O under continuous flow was carried out using this apparatus.

General Experimental Procedures

General Procedure A: Radical-Mediated Thiol-ene Reaction of Glutathione <u>in Batch</u>

Alkene (1.63 mmol), glutathione **1** (20 mg, 0.065 mmol) and photoinitiator (0.05 eq., 0.003 mmol) were stirred at rt in THF/H_2O (1 mL, 1:1) under UV irradiation (352 nm, 110 V) for 20 min. Solvent was removed *in vacuo*. Dimethyl sulfone (6 mg, 0.065 mmol) was added to the mixture and reaction conversions were measured using ^{1}H NMR spectroscopy with dimethyl sulfone as internal standard (1 eq.). ^{1}H NMR spectroscopic experiment were executed utilising a long relaxation time (D1 = 14 s). All compounds synthesised by this procedure were previously isolated by column chromatography and characterised to enable accurate determination of reaction conversions.

General Procedure B: Radical-Mediated Thiol-ene Reaction of Glutathione under <u>Continuous Flow</u>

A solution of glutathione **1** (20 mg, 0.065 mmol), alkene (0.16 mmol) and photoinitiator (0.05 eq., 0.03 mmol) in THF/H_2O (1 mL, 1:1) or H_2O (1 mL) was pumped at 80 µL min^{-1} with 9.99 mm syringe diameter through the coil reactor at room temperature under UV irradiation (352 nm, 110 V). Residence time was 20 min. Solvent was removed *in vacuo*. Dimethyl sulfone (6 mg, 0.065 mmol) was added to the mixture and reaction conversions were measured using ^{1}H NMR spectroscopy with dimethyl sulfone as internal standard (1 eq.). ^{1}H NMR spectroscopic experiment were executed utilising a long relaxation time (D1 = 14 s). All compounds

synthesised by this procedure were previously isolated by column chromatography and characterised to enable accurate determination of reaction conversions.

General Procedure C: Radical Mediated Thiol-ene Reactions of Glutathione in Deep Eutectic Solvents (DESs) and Bio-Based Solvents <u>in Batch</u>

Alkene (0.16 mmol), glutathione **1** (20 mg, 0.065 mmol) and photoinitiator (0.05 eq., 0.003 mmol) were stirred at rt in DES/H_2O (3:2, 1 mL) under UV irradiation (352 nm, 110 V) for 20 min. The resulting product containing solutions were collected and analysed by RP-HPLC.

General Procedure D: Radical Mediated Thiol-ene Reactions of Glutathione in Deep Eutectic Solvents (DES) and Bio-Based Solvents <u>under Continuous Flow</u>

A solution of glutathione **1** (20 mg, 0.065 mmol), alkene (0.16 mmol) and photoinitiator (0.05 eq., 0.03 mmol) in DES/H_2O (3:2, 1 mL) or H_2O (1 mL) was pumped at 80 µL min^{-1} with 9.99 mm syringe diameter through the coil reactor at room temperature under UV irradiation (352 nm, 110 V). Residence time was 20 min. Solvent was removed *in vacuo* and the resulting crude analysed by RP-HPLC.

General Procedure E: Solid Phase Fmoc Deprotection Utilising Wang Resin

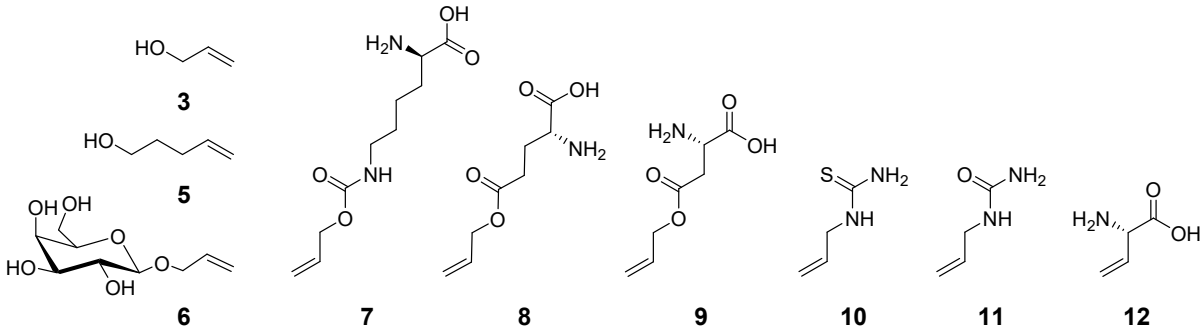
To a polypropylene syringe fitted with a polypropylene frit was added Wang resin (200 mg, 1.0 mmol/g, 0.2 mmol) and DMF (5 mL). The syringe was agitated for 20 min, then drained. To a solution of Fmoc-AA-OH (5 eq., 1.00 mmol) in DMF (1.5 mL) were added DIC (5 eq, 1.0 mmol.), HOBt (5 eq., 1 mmol) and DMAP (0.5 eq, 0.1 mmol). The resulting solution was preactivated for 5 minutes prior to addition to the syringe. The syringe was agitated for 1.5 h. Excess reagents were drained from the syringe and the resin was washed with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL) again. Fmoc deprotection of the resin bound peptide was then accomplished by the addition of piperidine (20% v/v) in DMF (2 x 10 mL, 20 min). The deprotection solution was expelled from the syringe and the resin washed with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL). Cleavage and global deprotection of the resin bound amino acids was undertaken by addition of cleavage cocktail (TFA:TES:H_2O, 95:2.5:2.5 v/v/v, 5 mL) to the syringe which was tightly capped and agitated for 1.5 hours. The syringe was drained, and the filtrate was collected. The resin was washed with TFA (2 x 2.5 mL) and

washings combined with the initial filtrate. The solution was concentrated under a stream of N_2 and triturated in cold Et_2O prior to freeze drying to yield the desired amino acid derivative.

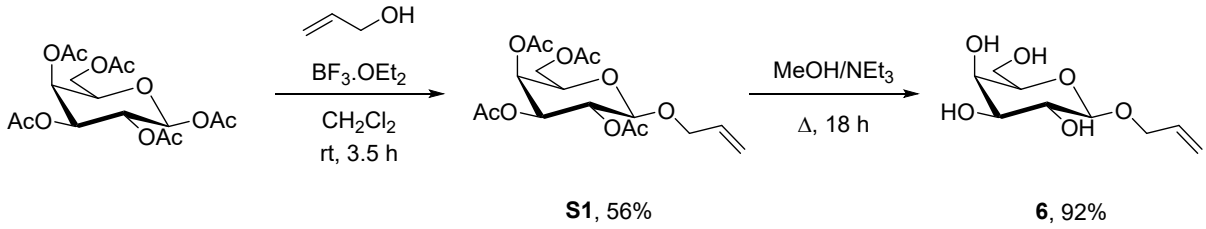
General Procedure F: Solid Phase Peptide Synthesis on Rink Amide Resin

To a polypropylene syringe fitted with a polypropylene frit was added Rink amide resin (256 mg, 0.78 mmol/g, 0.20 mmol), and DMF (5 mL). The syringe was agitated for 20 min, then drained. Fmoc deprotection of the resin was accomplished by the addition of piperidine (20% v/v) in DMF (2 x 10 mL, 20 min). The deprotection solution was expelled from the syringe and the resin washed with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL). PyBOP (3.0 eq.), NMM (6.0 eq.) and Fmoc-AA-OH protected amino acid (3.0 eq) were dissolved in DMF (0.2 M) and the resulting solution was preactivated for 5 minutes prior to addition to fritted syringe. The syringe was agitated for 45 min. Excess reagents were drained from the syringe and the resin was washed with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL). Subsequent amino acid coupling cycles consisted of i) Fmoc deprotection of the resin bound peptide by the addition of 20% (v/v) piperidine in DMF (5 mL) to the resin for 10 min. Following the solution was expelled from the syringe and replaced by fresh deprotection cocktail and the deprotection repeated, ii) resin washes with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL), iii) peptide coupling with addition of PyBOP (3.0 eq.), NMM (6.0 eq.) and Fmoc-AA-OH (3.0 eq) in DMF (0.2 M) to the peptide resin for 45 min, (iv) resin washes with DMF (3 x 5 mL), CH_2Cl_2 (3 x 5 mL) and DMF (3 x 5 mL), (v) qualitative monitoring of reaction progress with bromophenol blue. Following the final coupling, the resin was treated with 20% (v/v) piperidine in DMF twice for 10 min and the resin was washed with DMF (3 x 5 mL) and CH_2Cl_2 (3 x 5 mL). Cleavage and global deprotection of the resin bound amino acids was undertaken by addition of cleavage cocktail (TFA:TES:H_2O, 95:2.5:2.5 v/v/v, 5 mL) to the syringe which was tightly capped and agitated for 1.5 hours. The syringe was drained, and the filtrate was collected. The resin was washed with TFA (2 x 2.5 mL) and washings combined with the initial filtrate. The solution was concentrated under a stream of N_2 and triturated in cold Et_2O prior to freeze drying. The resulting crude peptide was purified by semi-preparative RP-HPLC.

Preparation of Alkene Starting Materials (3, 5 – 12)

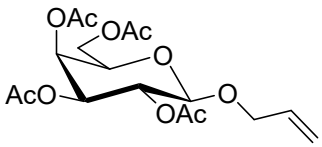


Allyl alcohol (**3**), pent-4-en-1-ol (**5**), allylthiourea (**9**) and allylurea (**10**) were purchased (Sigma Aldrich, Merck) and used in the radical mediated thiol-ene reactions described without further purification. (2R,3R,4S,5R,6R)-2-(allyloxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (**6**), N^6-((allyloxy)carbonyl)-D-lysine (**7**), (*R*)-5-(allyloxy)-2-amino-5-oxopentanoic acid (**8**), (*S*)-4-(allyloxy)-2-amino-4-oxobutanoic acid (**9**) and (*S*)-2-aminobut-3-enoic acid (**12**) were accessed synthetically, the details of which are outlined in the following section.



Scheme S1: Synthesis of Allyllated Monosaccharide **6**.

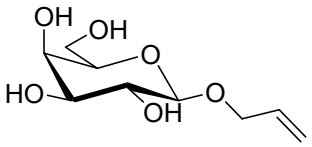
Allyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (S1).



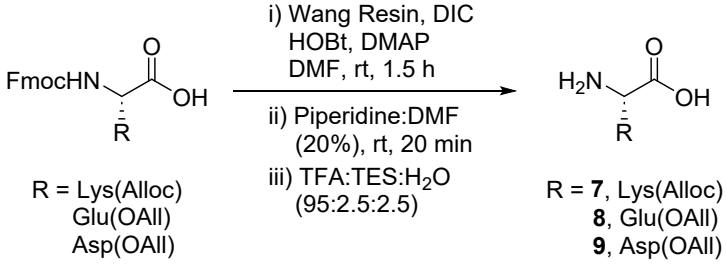
Peracetylated galactose (3.57 g, 9.15 mmol) was dissolved in dry CH_2Cl_2 (15 mL). To the mixture was added $BF_3{\cdot}OEt_2$ (1.24 mL, 10.06 mmol). The mixture was stirred for 1 h before addition of allyl alcohol **3** (0.93 mL, 13.7 mmol). The mixture was left stirring at rt for 3.5 h after which the reaction was quenched by addition of sat. aq. $NaHCO_3$ solution (20 mL). After stirring for 30 min, the mixture was washed three times with H_2O (3 × 20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash column

chromatography using Hex/EtOAc (6:4) as eluent afforded the product **S1** (2.00 g, 5.15 mmol, 56%) as a clear colourless syrup. Product spectroscopic data correlated to that reported in the literature.[1] $\mathbf{R_f}$ = 0.44 (Hex:EtOAc, 6:4).**^{1}H NMR** (600 MHz, $CDCl_3$): 5.84 (dddd, *J* = 17.0, 10.8, 6.3, 4.9 Hz, 1H, $OCH_2C\underline{H}=CH_2$), 5.27 (dd, *J* = 17.0, 1.8 Hz, 1H, $CH=C\underline{H}_A$), 5.22-5.18 (m, 2H, $CH=C\underline{H}_B$ + H3), 5.09 (app. t., *J* = 10,1 Hz, 1H, H4), 5.02 (dd, *J* = 9.6, 8.0 Hz, 1H, H2), 4.55 (d, *J* = 8.0 Hz, 1H, H1), 4.33 (app. ddt, *J* = 13.1, 4.9, 1.6 Hz, 1H, O-$C\underline{H}_{2C}$CH=CH2), 4.25 (dd, *J* = 12.2, 4.8 Hz, 1H, H6a), 4.14 (dd, *J* = 12.2, 2.4 Hz, 1H, H6b), 4.09 (app. ddt, *J* = 13.1, 6.3, 1.4 Hz, 1H, O-$C\underline{H}_{2D}$CH=CH2), 3.68 (ddd, *J* = 10.1, 4.8, 2.4 Hz, 1H, H5), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc)ppm. **HRMS**: (*m/z* ESI$^+$): found 411.1264 ([M+Na]$^+$, $C_{17}H_{24}NaO_{10}$ requires 411.1262).

(*2R*,3*R*,4*S*,5*R*,6*R*)-2-(allyloxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (6)

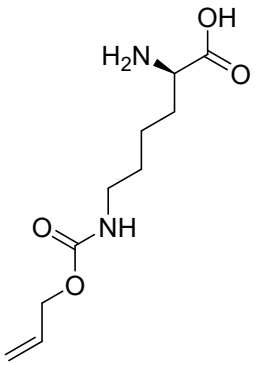


A round bottom flask was charged with compound **S1** (3.68 g, 9.48 mmol) and dissolved in MeOH/NEt_3 (4:1, 20 mL) and refluxed for 18 h. The solution was cooled down to rt and DOWEX (H+) 50WX8-200 resin was then added to the flask until pH = 5 was achieved. The resin was filtered off and the solvent was evaporated *in vacuo* to yield a red light solid. CH_2Cl_2 (20 mL) was then added, the resulting solid was isolated *via* filtration under reduced pressure. The filtered solid was dissolved in MeOH. Finally, the solvent was removed *in vacuo*. Compound **6** was obtained as a white solid (1.91 g, 92%). Product spectroscopic data correlated well to that in the literature.[2] **m.p** 104-106 °C (Lit.[3] m.p. 102-103). $\mathbf{R_f}$ = 0.82 (IPA:ACN:H_2O, 10:9:2). **^{1}H NMR** (400 MHz, MeOD-d_4) δ 6.00 (dddd, *J* = 17.0, 10.9, 6.1, 5.2 Hz, 1H,$OCH_2C\underline{H}=CH_2$), 5.36 (dd, *J* = 17.0, 1.7 Hz, 1H, $OCH_2CH=C\underline{H}_{2A}$), 5.19 (dd, *J* = 10.9, 1.7 Hz, 1H, $OCH_2CH=C\underline{H}_{2B}$), 4.41 (app. ddt, *J* = 12.8, 5.2, 1.6 Hz, 1H, $OC\underline{H}_{2C}CH=CH_2$), 4.33 (d, *J* = 7.8 Hz, 1H, H1), 4.18 (app. ddt, *J* = 12.8, 6.1, 1.6 Hz, 1H, $OC\underline{H}_{2D}CH=CH_2$), 3.89 (dd, *J* = 11.9, 2.0 Hz, 1H, H6a), 3.69 (dd, *J* = 11.9, 5.3 Hz, 1H, H6b), 3.38 - 3.27 (m, 3H, H3, H4, H5), 3.23z (dd, *J* = 9.0, 7.8 Hz, 1H, H2). ppm **HRMS** (*m/z* ESI$^+$): found 243.0837 ([M+Na]$^+$, $C_9H_{16}NaO_6$ requires 243.0839).



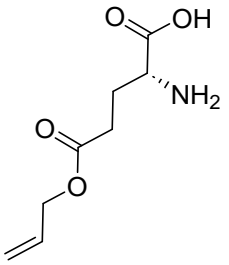
Scheme S2: Deprotection of allylated amino acids **7**, **8** and **9**.

N^6-((allyloxy)carbonyl)-D-lysine (7)



7 was prepared by solid phase Fmoc deprotection of Fmoc-Lys(Alloc)-OH as per **general procedure E** (138 mg, 40%) and was obtained as a white solid. Product spectroscopic data correlated well to that in the literature.[3] **m.p.** 191-199 °C (dec.) (Lit.[4] m.p. 182-190 °C). **^{1}H NMR** (400 MHz, D_2O): δ 5.96 (ddt, *J* = 17.0, 10.5, 5.0 Hz, 1H, C<u>H</u>=CH_2), 5.33 (d, *J* = 17.4 Hz, 1H, CH=C<u>H</u>$_{2A}$), 5.26 (d, *J* = 10.6 Hz, 1H, CH=C<u>H</u>$_{2B}$), 4.57 (d, *J* = 5.0 Hz, 2H, OC<u>H</u>$_2$CH=CH_2), 4.00 (t, *J* = 6.3 Hz, 1H, Lys αCH), 3.17 (t, *J* = 6.6 Hz, 2H, Lys βCH_2), 2.05-1.87 (m, 2H, Lys εCH_2), 1.57 (app. p, *J* = 6.2 Hz, 2H, Lys δCH_2), 1.51-1.40 (m, 2H, Lys γCH_2) ppm. **HRMS** (*m/z* ESI$^+$); found 231.1235 (M+H]$^+$, $C_{10}H_{18}N_2O$ requires 231.1267).

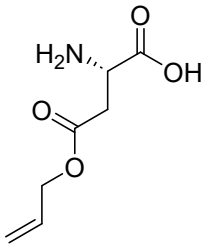
(*S*)-5-(allyloxy)-2-amino-5-oxopentanoic acid (8)



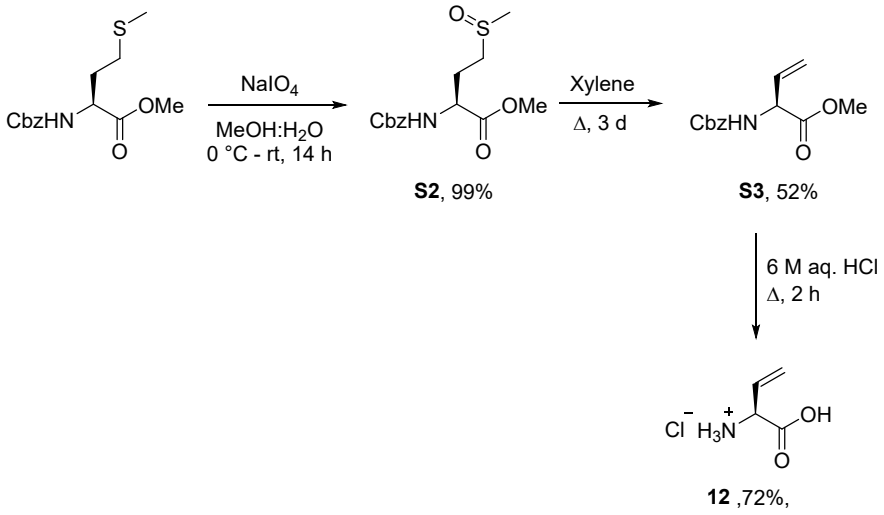
8 was prepared by solid phase Fmoc deprotection of Fmoc-Glu(OAll)-OH as per **general procedure E** (92 mg, 40%) and was obtained as a yellow oil. Product spectroscopic data correlated well to that in the literature.[3] **^{1}H NMR** (400 MHz, D_2O): δ 5.99 (ddt, *J* = 16.7, 11.1,

5.7 Hz, 1H, $OCH_2C\underline{H}=CH_2$), 5.38 (dd, *J* = 16.7, 1.6 Hz, 1H, $OCH_2CH=C\underline{H}_{2A}$), 5.31 (dd, *J* = 11.1, 1.6 Hz, 1H, $CH=C\underline{H}_{2B}$), 4.66 (dt, *J* = 5.7, 1.5 Hz, 2H, $OC\underline{H}_2CH=CH_2$), 3.89 (td, *J* = 6.5, 1.8 Hz, 1H, Glu αCH), 2.67-2.62 (m, 2H, Glu γCH_2), 2.29-2.14 (m, 2H, Glu βCH_2) ppm. **HRMS** (*m/z* ESI^+): found 188.0880 ($[M+H]^+$, $C_8H_{13}NO_4$ requires 188.0845).

(*R*)-4-(allyloxy)-2-amino-4-oxobutanoic acid (9)

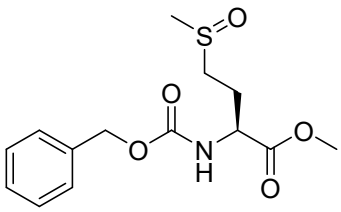


9 was prepared by solid phase Fmoc deprotection of Fmoc-Asp(OAll)-OH as per **general procedure E** (146 mg, 53%) and was obtained as a white solid. Product spectroscopic data correlated well to that in the literature.[3] **m.p.** 100-105 °C (dec.) (Lit.[4] m.p. 105-108 °C). **^{1}H NMR** (400 MHz, D_2O): δ 5.41 (ddd, *J* = 17.4, 11.0, 6.0 Hz, 1H, $C\underline{H}=CH_2$), 5.34 (d, *J* = 17.4 Hz, 1H, $CH=C\underline{H}_{2A}$), 5.34 (d, *J* = 11.0 Hz, 1H, $CH=C\underline{H}_{2B}$), 4.50 (app. t, *J* = 6.0 Hz, 2H, $OC\underline{H}_2CH=CH_2$), 3.23 (dd, *J* = 18.4, 5.9 Hz, 1H, Asp βCH_2), 3.23 (dd, *J* = 18.4, 4.3 Hz, 1H, Asp βCH_2), 3.03-3.02 (m, 1H,Asp αCH) ppm. **HRMS** (*m/z* ESI^+): found 174.0625 ($[M+H]^+$, $C_7H_{11}NO_4$ requires 174.0688).



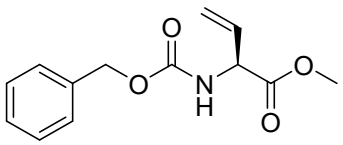
Scheme S3: Synthesis of vinylglycine **12**.

Methyl (2*S*)-2-(((Benzyloxy)carbonyl)amino)-4-(methylsulfinyl)butanoate (S2)



To stirred solution of Cbz-Met-OMe (5.00 g, 16.9 mmol) in MeOH (25 mL) at 0 °C was added dropwise a solution of $NaIO_4$ (3.96 g, 18.6 mmol) in H_2O (25 mL) after which the reaction was allowed to reach rt and stirred for 14 h. The resulting mixture was filtered through celite to remove precipitated solids and the filtrate extracted with $CHCl_3$ (3 x 25 mL). Combined extracts were washed with brine (2 x 30 mL) and dried over $MgSO_4$ to yield the product **S2** as a pale yellow oil (5.20 g, 99%). The isolated compound was in good agreement with the literature.[5] **^{1}H NMR** (400 MHz, $CDCl_3$): 7.37 – 7.28 (m, 5H, Ar-CH), 5.74 (dd, *J* = 27.9, 7.7 Hz, 1H, NH), 5.08 (s, 2H, Ar-CH_2CO), 4.52 – 4.42 (m, 1H, αCH), 3.74 (s, 3H, OCH_3), 2.81 – 2.63 (m, 2H, γCH_2), 2.53 (d, *J* = 3.0 Hz, 3H, $SOCH_3$), 2.44 – 2.27 (m, 1H, βCH_2), 2.21 – 2.03 (m, 1H, βCH_2) ppm **HRMS** (*m/z* ESI$^+$): Found: 336.0879 ([M + Na]$^+$, $C_{14}H_{19}NO_5Na$ requires 336.0876).

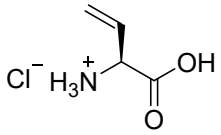
Methyl *(S)*-2-



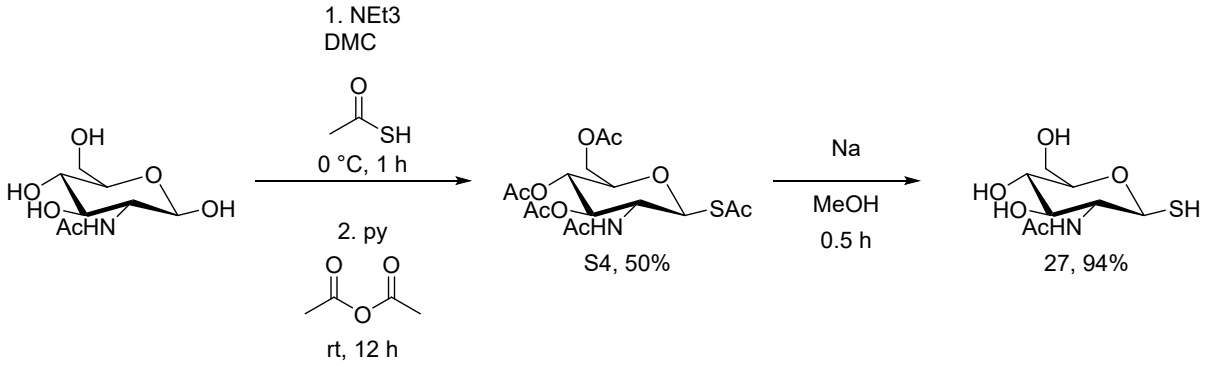
(((benzyloxy)carbonyl)amino)but-3-enoate (S3)

A stirred solution of **S2** (5.00 g, 20.1 mmol) in xylene (50 mL) was heated under reflux for 72 h. Solvent was evaporated *in vacuo* and the resulting brown residue was purified by silica gel flash chromatography (Hex:EtOAc, 9:1 to 8:2) to yield the product **S3** as a colourless oil (2.08 g, 52%). The isolated compound was in good agreement with the literature.[5] **^{1}H NMR** (400 MHz, $CDCl_3$): 7.37 – 7.28 (m, 5H, Ar-CH), 5.74 (dd, J = 27.9, 7.7 Hz, 1H, NH), 5.08 (s, 2H, Ar-CH_2CO), 4.52 – 4.42 (m, 1H, αCH), 3.74 (s, 3H, OCH_3), 2.81 – 2.63 (m, 2H, γCH_2), 2.53 (d, J = 3.0 Hz, 3H, $SOCH_3$), 2.44 – 2.27 (m, 1H, βCH_2), 2.21 – 2.03 (m, 1H, βCH_2) ppm **HRMS** (m/z ESI+): Found: 336.0879 ([M + Na]+, C14H19NO5Na requires 336.0876).

(S)-2-Aminobut-3-enoic acid hydrochloride (12)

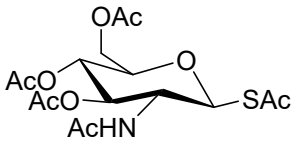


A solution of **S3** (1.00 g, 4.01 mmol) in 6 M aq. HCl solution (20 mL) was heated under reflux for 1.5 h. After cooling, the resulting mixture was washed with $CHCl_3$ (2 x 15 mL) and solvent removed *in vacuo* to yield the crude product. Recrystallisation from acetone gave the product **12** as a white crystalline solid in a 72% yield. The isolated compound was in good agreement with the literature.[5] **^{1}H NMR** (400 MHz, D_2O): δ 5.85 (ddd, *J* = 17.5, 10.4, 7.4 Hz, 1H, C<u>H</u>=CH_2), 5.45 (dd, *J* = 17.5, 1.3 Hz, 1H, CH=C<u>H_{2A}</u>), 5.41 (dd, *J* = 10.4, 1.3 Hz, 1H, CH=C<u>H_{2B}</u>), 4.43 (d, *J* = 7.4 Hz, 1H, αCH) ppm. **HRMS** (*m/z* ESI^+): found 102.0552 ($[M+H]^+$, $C_4H_8NO_2$ requires 102.0550).



Scheme S4: Synthesis of glycosylated thiol **27**

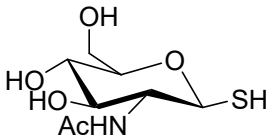
1-thioacetyl-3,4,6-tri-Oacetyl-2-deoxy-2-acetamido-β-D-glucopyranoside (S4).



N-Acetyl-D-glucosamine (500 mg, 2.26 mmol) and triethylamine (3.1 mL, 22.6 mmol) were stirred in water (10 mL) and cooled to 0 °C. 2-Chloro-1,3-dimethylimidazolinium chloride (1.2 g, 6.78 mmol) was added. After 0.5 h, thioacetic acid (2.4 mL, 33.9 mmol) was added dropwise, and the reaction mixture was allowed to stir for an additional 0.5 h at 0 °C. The reaction mixture was then diluted with water (10 mL) and washed with CH_2Cl_2 (5 x 20 mL). The aqueous layer

was concentrated in vacuo and the solid suspended in pyridine (7 mL) under an atmosphere of nitrogen. The mixture was stirred at rt and acetic anhydride (7 mL, 74.1 mmol) was added. The reaction was stirred for 12 h, after which time TLC (100% EtOAc) indicated the formation of a single major product. Water (10 mL) was added, and the mixture was then washed with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were washed with aqueous HCl (1 M, 100 mL), $NaHCO_3$ (sat. aqueous soln., 100 mL), water (100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was recrystallised (Hex/CH_2Cl_2) to afford 1-thioacetyl-3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside **S4** (453 mg, 50%) as a white solid. The isolated compound was in good agreement with the literature.[6] **m.p** = 180-186 °C (Lit.[5] m.p. 186-188 °C). Rf = 0.53 (100% EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (d, *J* = 9.7 Hz, 1H, NH), 5.3 – 5.0 (m, 3H, H1+ H3 + H4), 4.35 (app. q, *J* = 10.1 Hz, 1H), 4.24 (dd, *J* = 12.5, 4.5 Hz, 1H. H6a), 4.10 (dd, *J* = 12.4, 2.1 Hz, 1H, H6b), 3.80 – 3.76 (m, 1H, H5), 2.4 (s, 3H,OAc), 2.1 (s, 3H, OAc), 2.0 (s, 6H, OAc), 1.9 (s, 3H, OAc) ppm. **HRMS** (*m/z* ESI$^+$): found 406.116823 ([M+H]$^+$, $C_{16}H_{24}NO_9S$ requires 406.116629).

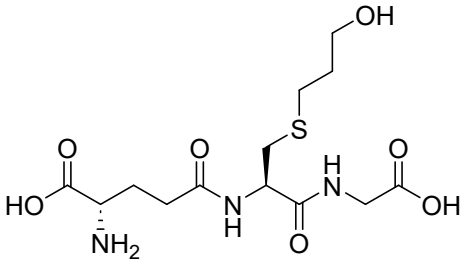
N-((2S,3R,4R,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-2-mercaptotetrahydro-2H-pyran-3-yl)acetamide (27).



Sodium metal (25.4 mg, 1.1 mmol) was added to MeOH (5 mL) and stirred. 1-Thioacetyl3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-β-D-glucopyranoside **S4** (203 mg, 0.50 mmol) was added and the reaction was stirred for 0.5 h, at which point TLC (100% EtOAc) indicated complete consumption of starting material (Rf = 0.53) and formation of a single product (Rf = 0). Dowex® 50WX8 (H+) ion exchange resin was added portion-wise until the reaction reached neutral pH. The mixture was then filtered and concentrated *in vacuo* to afford 1-thio-2-acetamido-2-deoxy-β-D-glucopyranose **27** (121 mg, 94%) as a white solid. The isolated compound was in good agreement with the literature.[6] **m.p.** 175-178 °C [lit.[5] 177-179 °C]; ^{1}H NMR (400 MHz, $MeOD_4$) δ 4.57 (d, *J* = 10.0 Hz, 1H, H1), 3.88 (dd, *J* = 12.0, 2.0 Hz, 1H, H6a), 3.74 – 3.65 (m, 3H, H2 + H6b), 3.45 – 3.36 (m, 3H. H 3+ H4+ H5), 2.0 (s, 3H, OAc) ppm. **HRMS** (*m/z* ESI$^+$): found 238.0746 ([M+H]$^+$, $C_8H_{16}NO_5S$ requires 238.0744).

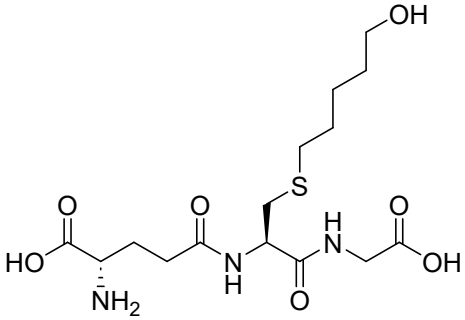
Characterisation Data of Thiol-ene Products (4, 13 – 20)

N-((*R*)-1-((carboxymethyl)amino)-3-((3-hydroxypropyl)thio)-1-oxopropan-2-yl)-D-glutamine (4)



4 was prepared by radical mediated thiol-ene reaction of glutathione **(1)** and allyl alcohol (**3)** as per **general procedure A** in batch (21 mg, 89%) and as per **general procedure B** under continuous flow (22 mg, 91%). Upon completion, the reaction mixture was washed with CH_2Cl_2 (2 x 50 mL), and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. Product spectroscopic data correlated well to that in the literature.[7] **m.p.** 131-139 °C (dec.). $\mathbf{R_f}$ = 0.59 (IPA:ACN:H_2O, 10:9:2).**^{1}H NMR** (400 MHz, D_2O): δ 4.59 (dd, *J* = 8.7, 5.1 Hz, 1H, Cys αCH), 3.96 (s, 2H, Gly αCH_2), 3.82 (t, *J* = 6.3 Hz, 1H, Glu αCH), 3.68 (t, *J* = 6.3 Hz, 2H, $CH_2CH_2C\underline{H}_2OH$), 3.09 (dd, *J* = 14.1, 5.1 Hz, 1H, Cys βCH_2), 2.90 (dd, *J* = 14.1, 8.7 Hz, 1H, Cys βCH_2), 2.67 (t, *J* = 7.5, Hz, 2H, Glu γCH_2), 2.58-2.52 (m, 2H, Glu βCH_2),), 2.21-2.15 (m, 2H, $\underline{CH_2}CH_2CH_2OH$), 1.88 – 1.79 (m, 2H,$CH_2\underline{CH_2}CH_2OH$) ppm. **HRMS** (*m/z* ESI$^+$): found 366.1329 ([M+H]$^+$, $C_{13}H_{24}N_3O_7S$ requires 366.1329).

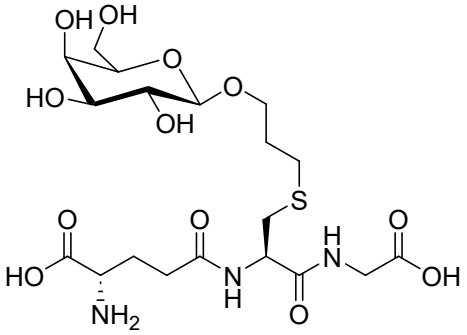
N-((R)-1-((carboxymethyl)amino)-3-((5-hydroxypentyl)thio)-1-oxopropan-2-yl)-D-glutamine (13)



13 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and 4-penten-1-ol (**5**) as per **general procedure A** in batch (21 mg, 84%) and as per **general procedure B** under continuous flow (24 mg, 94%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 180-185 °C

(dec.). $\mathbf{R_f}$ = 0.56 (IPA:ACN:H_2O, 10:9:2).**^{1}H NMR** (400 MHz, D_2O): δ 4.59 (dd, *J* = 8.7, 5.1 Hz, 1H, Cys αCH), 4.00 (s, 2H, Gly αCH_2), 3.85 (t, *J* = 6.4 Hz, 1H, Glu αCH), 3.62 (t, *J* = 6.5 Hz, 2H, $CH_2CH_2CH_2CH_2C\underline{H}_2OH$), 3.08 (dd, *J* = 14.0, 5.1 Hz, 1H, Cys βCH_2), 2.90 (dd, *J* = 14.0, 8.7 Hz, 1H, Cys βCH_2), 2.63 (t, *J* = 7.4 Hz, 1H, Glu γCH_2), 2.59-2.54 (m, 2H, Glu βCH_2), 2.22-2.16 (m, 2H, $C\underline{H}_2CH_2CH_2CH_2CH_2OH$), 1.64 (dt, *J* = 8.5, 6.8 Hz, 2H,), 1.64-1.54 (m, 4H, $CH_2\underline{CH}_2CH_2CH_2CH_2OH$ + $CH_2CH_2CH_2\underline{CH}_2CH_2OH$), 1.49-1.37 (m, 2H, $CH_2CH_2\underline{CH}_2CH_2CH_2OH$) ppm. **^{13}C NMR** (101 MHz, D_2O): δ 174.8 (C=O), 173.5 (C=O), 173.5 (C=O), 172.8 (C=O), 61.6 ($CH_2CH_2CH_2CH_2\underline{C}H_2OH$), 54.8 (Glu αCH), 53.2 (Cys αCH), 41.3 (Gly αCH_2), 32.7 (Cys βCH_2), 31.5 (Glu γCH_2), 31.2 (Glu βCH_2), 30.8 ($CH_2CH_2CH_2\underline{C}H_2CH_2OH$), 27.9 ($CH_2\underline{C}H_2CH_2CH_2CH_2OH$), 26.0 ($\underline{C}H_2CH_2CH_2CH_2CH_2OH$) 24.2 ($CH_2CH_2\underline{C}H_2CH_2CH_2OH$) ppm. **HRMS** (*m/z* ESI$^+$): found 392.1487 ([M+H]$^+$, $C_{15}H_{26}N_3O_7S$ requires 392.1496). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3342 (N-H, O-H), 2929 (CH_2), 1673 (C=O), 1256 (C-O), 684 (C-S).

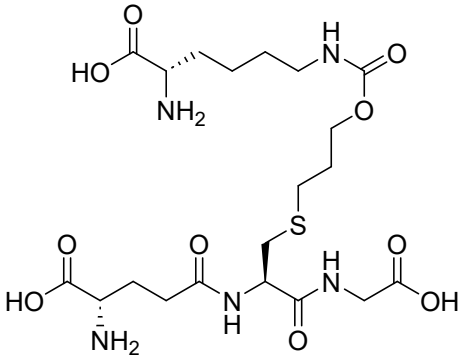
***N*-((*R*)-1-((carboxymethyl)amino)-1-oxo-3-((3-(((2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propan-2-yl)-L-glutamine (14)**



14 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and allylated monosaccharide **6** as per **general procedure A** in batch (34 mg, 96%) and as per **general procedure B** under continuous flow (49 mg, 94%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 132-140 °C (dec.). $\mathbf{R_f}$ = 0.50 (EtOH:H_2O, 8:2).**^{1}H NMR** (400 MHz, D_2O): δ 4.59 (dd, *J* = 8.9, 4.9 Hz, 1H, Cys αCH), 4.46 (d, *J* = 7.9 Hz, 1H, H1), 4.02-3.97 (m, 1H, Glu αCH), 3.92 (dd, *J* = 12.4, 2.2 Hz, 1H, H6a), 3.79-3.73 (m, 5H, Gly αCH_2 + $C\underline{H}_{2A}CH_2CH_2S$ + $C\underline{H}_{2B}CH_2CH_2S$+ H5), 3.72-3.62 (m, 1H, H6b), 3.52-3.44 (m, 1H, H4), 3.40-3.36 (m, 1H, H3), 3.27 (dd, *J* = 9.3, 7.9 Hz, 1H, H2), 3.10 (dd, *J* = 14.1, 5.0 Hz, 1H, Cys βCH_2), 2.89 (dd, *J* = 14.1, 8.9 Hz, 1H, Cys βCH_2), 2.70 (t, *J* = 6.9 Hz, 2H, Glu γCH_2), 2.55 (app. td, *J* = 7.5, 2.5 Hz, 2H, $CH_2CH_2C\underline{H}_2S$), 2.20-2.14 (m, 2H, $CH_2C\underline{H}_2CH_2S$), 1.96-1.88 (m, 2H, Glu βCH_2) ppm.**^{13}C**

NMR (101 MHz, D_2O): δ 176.2 (C=O), 174.9 (C=O), 174.3 (C=O), 171.7 (C=O), 102.3 (C1), 75.7 (C3), 73.1 (C2), 69.7 (C4), 68.71 (Glu αCH, C$\underline{H}_2$CH$_2$CH$_2$S), 60.6 (CH_2, C6), 54.1 (C5), 53.9 (CH$_2$CH$_2$C$\underline{H}_2$O), 53.2 (Cys αCH), 43.3 (Gly αCH_2), 32.8 (Cys βCH_2), 31.4 (Glu βCH_2), 28.8 (C$\underline{H}_2$CH$_2$CH$_2$O), 27.9 (Glu γCH_2), 26.2 (CH$_2$C$\underline{H}_2$CH$_2$O) ppm. **HRMS** (*m/z* ESI$^+$): found 550.1674 ([M+Na]$^+$, $C_{19}H_{33}N_3NaO_{12}S$ requires 550.1677). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3263 (N-H), 2929 (CH_2), 1634 (C=O), 1235 (C-O).

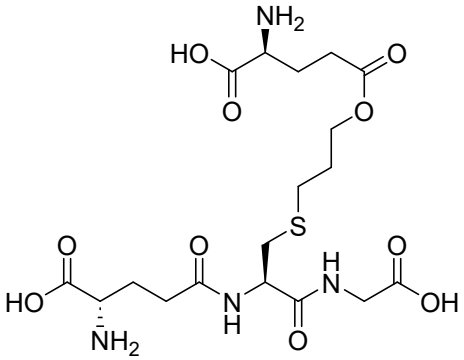
(2*R*, 7*R*, 20*R*)-2,20-diamino-7-((carboxymethyl)carbamoyl)-5,14-dioxo-13-oxa-9-thia-6,15-diazahenicosanedioic acid (15)



15 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and Lys(OAll)-OH **7** as per **general procedure A** in batch (55%) and as per **general procedure B** under continuous flow (75%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 187-195 °C (dec.). $\mathbf{R_f}$ = 0.66 (H_2O:ACN, 7:3) reverse phase C_{18} TLC.**^{1}H NMR** (600 MHz, DMSO-d_6): δ 8.19 (d, *J* = 6.0 Hz, 1H, Gly NH), 8.05 (d, *J* = 8.5 Hz, 1H, Cys NH), 6.85 (t, *J* = 5.8 Hz, 1H, Lys NH), 4.23 (ddd, J = 9.4, 8.5, 4.8 Hz, 1H, Cys αCH), 3.74 (t, *J* = 6.5 Hz, 2H, SCH$_2$CH$_2$C$\underline{H}_2$O), 3.66 (app. t, *J* = 6.4 Hz, 1H, Glu αCH), 3.60 (app. t, *J* = 6.2 Hz, 1H, Lys αCH), 3.53 (d, *J* = 6.0 Hz, 2H, Gly αCH_2), 2.74-2.72 (m, 2H, Lys εCH_2), 2.63 (dd, *J* = 13.7, 4.8 Hz, 1H, Cys βCH_2), 2.38 (dd, *J* = 13.6, 9.4 Hz, 1H, Cys βCH_2), 2.34-2.30 (m, 2H, SC$\underline{H}_2$CH$_2$CH$_2$O), 2.16 (ddd, *J* = 15.3, 9.4, 6.2 Hz, 1H, Glu γ_1CH_2), 2.08 (ddd, *J* = 15.3, 9.3, 6.2 Hz, 1H, Glu γ_2CH_2), 1.85-1.70 (m, 2H, Glu βCH_2), 1.57-1.47 (m, 4H, SCH$_2$C$\underline{H}_2$CH$_2$O+ Lys βCH_2), 1.20-1.12 (m, 2H, Lys γCH_2), 1.09-1.03 (m, 2H, Lys δCH_2) ppm.**^{13}C NMR** (151 MHz, DMSO): δ 171.1 (C=O), 171.1 (C=O), 170.9 (C=O, Lys), 170.8 (C=O), 170.6 (C=O), 156.2 (C=O), 62.3 (SCH$_2$CH$_2$C$\underline{H}_2$O), 52.1 (Cys αCH), 52.0 (Lys αCH), 51.8 (Glu αCH), 40.7 (Gly αCH_2), 39.8 (Lys εCH_2), 33.5 (Cys βCH_2), 30.7 (Glu γCH_2), 29.7 (Lys βCH_2), 28.8 (Lys γCH_2), 28.6 (SCH$_2$C$\underline{H}_2$CH$_2$O), 27.7

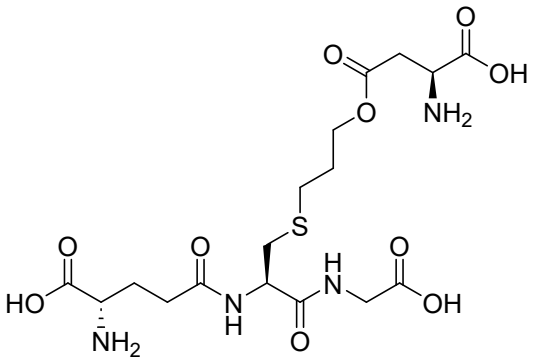
(S<u>C</u>$H_2CH_2CH_2O$), 26.1 (Glu βCH_2), 21.6 (Lys δCH_2) ppm. **HRMS** (*m/z* ESI^+): found 538.2184 ($[M+H]^+$, $C_{20}H_{36}N_5O_{10}S$ requires 538.2177). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3320 (N-H), 2939 (CH_2), 1661 (C=O), 1241 (C-O).

(*R*)-2-amino-5-(3-(((*R*)-2-((*R*)-4-amino-4-carboxybutanamido)-3-((carboxymethyl)amino)-3-oxopropyl)thio)propoxy)-5-oxopentanoic acid (16)



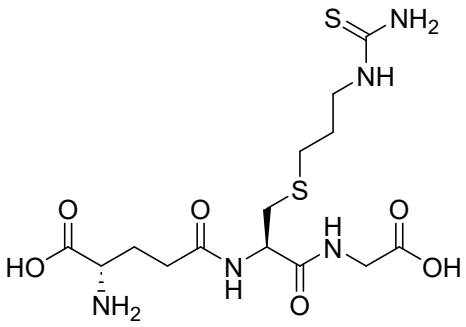
16 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and Glu(OAll)-OH **8** as per **general procedure A** in batch (73%) and as per **general procedure B** under continuous flow (86%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^1H NMR spectroscopic analysis. **m.p.** 120-135 °C (dec.). $\mathbf{R_f}$ = 0.94 (H_2O:ACN, 9:1) reverse phase C_{18} TLC. **^{1}H NMR** (600 MHz, DMSO-d_6): δ 8.43 (t, *J* = 5.7 Hz, 1H, Gly NH), 8.27 (d, *J* = 8.5 Hz, 1H, Cys NH), 4.48 (ddd, *J* = 8.9, 8.5, 4.8 Hz, 1H, Cys αCH), 3.98 (t, *J* = 6.4 Hz, 2H, Glu αCH + Glu (GSH) αCH), 3.90-3.83 (m, 2H, $SCH_2CH_2C\underline{H}_2O$), 3.76 (dd, *J* = 5.7, 2.2 Hz, 2H, Gly αCH_2), 2.96 (app. q, *J* = 6.6 Hz, 2H, $SC\underline{H}_2CH_2CH_2O$), 2.87 (dd, *J* = 13.6, 4.8 Hz, 1H, Cys βCH_2), 2.64-2.60 (dd, *J* = 13.6, 8.9 Hz, 1H, Cys βCH_2), 2.57-2.54 (m, 2H, Glu (OAll) βCH_2), 2.40-2.29 (m, 2H, Glu (GSH) βCH_2), 1.80-1.72 (m, 4H, Glu(GSH) γCH_2+ Glu γCH_2), 1.43-1.37 (m, 2H, $SCH_2C\underline{H}_2CH_2O$) ppm.**^{13}C NMR** (151 MHz, DMSO-d_6): δ 207.1 (C=O), 171.6 (2 C=O), 171.44 (C=O), 171.40 (C=O), 171.1 (C=O), 62.8 (Glu αCH), 52.6 (Cys αCH), 52.5 ($SCH_2CH_2\underline{C}H_2O$), 41.2 (Gly α$CH_2$), 40.4 (S<u>C</u>$H_2CH_2CH_2O$), 34.1 (Cys β$CH_2$), 30.2 (Glu γ$CH_2$), 29.3 (Glu (GSH) β$CH_2$), 29.2 (Glu β$CH_2$), 28.20 (Glu (OAll) γ$CH_2$), 22.1 ($SCH_2\underline{C}H_2CH_2O$) ppm. **HRMS** (*m/z* ESI^+): found 495.1757 ($[M+H]^+$, $C_{18}H_{31}N_4O_{10}S$ requires 495.1755). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 2936 (CH_2), 1635 (C=O), 1199 (C-O).

***N*-((*R*)-3-((3-(((*S*)-3-amino-3-carboxypropanoyl)oxy)propyl)thio)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-L-glutamine (17)**



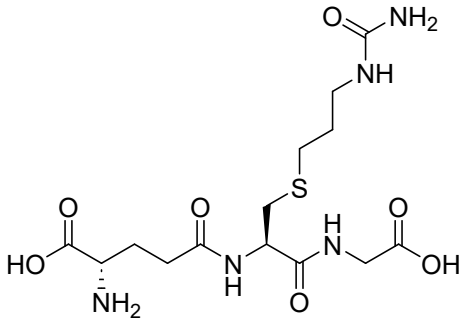
17 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and Asp(OAll)-OH **9** as per **general procedure A** in batch (53%) and as per **general procedure B** under continuous flow (83%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 131-139 °C (dec.). $\mathbf{R_f}$ = 0.67 (H_2O:ACN, 9:1) reverse phase C_{18} TLC.**^{1}H NMR** (400 MHz, D_2O): δ 4.60 (dt, *J* = 8.7, 4.1 Hz, 1H, Asp αCH), 4.49-4.42 (m, 1H, Cys αCH), 4.40 (dd, *J* = 8.9, 7.5 Hz, 1H, Asp βCH_2), 4.35 (dd, *J* = 7.5, 4.1 Hz, 1H, Asp βCH_2), 4.01 (s, 2H, Gly αCH_2), 3.89 (t, *J* = 5.7 Hz, 1H, Glu αCH), 3.20 (dd, *J* = 18.2, 5.8 Hz, 1H, Cys βCH_2), 3.13-3.01 (m, 1H, Cys βCH_2), 2.94-2.87 (m, 2H, C$\underline{H}_2CH_2CH_2O$), 2.69 (t, *J* = 7.3 Hz, 2H, $CH_2CH_2C\underline{H}_2O$), 2.60-2.55 (m, 2H, Glu βCH_2), 2.23-2.17 (m, 2H, Glu γCH_2), 2.05-1.95 (m, 2H, $CH_2C\underline{H}_2CH_2O$) ppm.**^{13}C NMR** (101 MHz, D_2O): δ 174.8 (C=O), 173.6 (C=O), 173.3 (C=O), 172.8 (C=O), 169.2 (C=O), 65.7 (Asp βCH_2), 53.5 (Glu αCH), 53.0 (Asp αCH), 49.5 (Cys αCH), 41.3 (Gly αCH_2), 34.1 (Cys βCH_2), 32.7 ($\underline{C}H_2CH_2CH_2O$), 31.1 (Glu β$CH_2$), 27.9 ($CH_2CH_2\underline{C}H_2O$), 27.4 ($CH_2\underline{C}H_2CH_2O$), 25.9 (Glu γ$CH_2$) ppm. **HRMS** (*m/z* ESI$^+$): found 481.1599, ([M+H]$^+$, $C_{17}H_{29}N_4O_{10}S$ requires 481.1598). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3338 (N-H), 2929 (CH_2), 1671 (C=O), 1222 (C-O).

***N*-((*R*)-1-((carboxymethyl)amino)-1-oxo-3-((3-thioureidopropyl)thio)propan-2-yl)-L-glutamine (18)**



18 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and *N*-allylthiourea **10** as per **general procedure A** in batch (quantitative) and as per **general procedure B** under continuous flow (84%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 210-215 °C (dec.). $\mathbf{R_f}$ = 0.61 (H_2O:ACN, 9:1) reverse phase C_{18} TLC.**^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.53 (d, *J* = 9.6, 5.3 Hz, 1H, Gly NH), 8.34 (d, *J* = 8.6 Hz, 1H, Cys NH), 7.08 (t, *J* = 5.8 Hz, 1H, Thiourea NH), 4.46 (ddd, *J* = 8.7, 8.61, 4.2 Hz, 1H, Cys αCH), 3.97 (t, *J* = 6.3 Hz, 1H, Glu αCH), 3.73 (d, *J* = 5.6 Hz, 2H, Gly αCH_2), 3.60-3.53 (m, 2H, S$\underline{C}H_2CH_2CH_2NH$), 2.88 (dd, *J* = 13.5, 4.6 Hz, 1H, Cys βCH_2), 2.62 (dd, *J* = 13.8, 8.61 Hz, 1H, Cys βCH_2), 2.55 (t, *J* = 7.2 Hz, 1H, Glu γCH_2), 2.37-2.31 (m, 2H, $SCH_2C\underline{H}_2CH_2NH$), 1.97-1.89 (m, 2H, $SCH_2CH_2C\underline{H}_2NH$), 1.78-1.70 (m, 2H, Glu βCH_2) ppm.**^{13}C NMR** (101 MHz, DMSO-d_6): δ 172.1 (C=O), 172.1 (C=O), 170.8 (C=O), 170.8 (C=O), 156.7 (C=S), 62.7 (Glu αCH), 55.5 (Cys αCH), 53.4 ($S\underline{C}H_2CH_2CH_2NH$), 52.9 (Cys αCH), 41.4 (Gly αCH_2), 34.1 (Cys βCH_2), 31.9 ($SCH_2CH_2\underline{C}H_2NH$), 29.3 (Glu β$CH_2$), 28.3 (Glu γ$CH_2$), 27.2 ($SCH_2\underline{C}H_2CH_2NH$) ppm. **HRMS** (*m/z* ESI$^+$): found 424.1319 ([M+H]$^+$, $C_{14}H_{26}N_5O_6S_2$ requires 424.1319). $\mathbf{\nu_{max}}$ (film)/cm^{-1}: 2937 (CH_2), 1645 (C=O), 1133 (C=S).

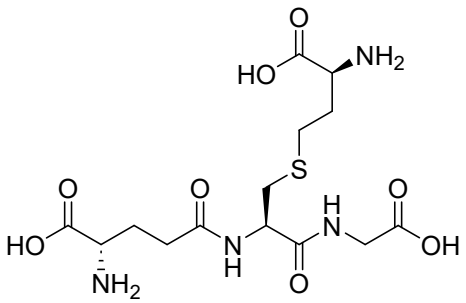
_N_-((_R_)-1-((carboxymethyl)amino)-1-oxo-3-((3-ureidopropyl)thio)propan-2-yl)-L-glutamine (19)



19 was prepared by radical mediated thiol-ene reaction of glutathione **25** and *N*-allylurea **11** as per **general procedure A** in batch (36%) and as per **general procedure B** under continuous flow (67%) as a white solid. The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 195-200 °C (dec.). $\mathbf{R_f}$ = 0.88 (H_2O:ACN, 9:1) reverse phase C_{18} TLC.**^{1}H NMR** (400 MHz, DMSO-d_6): δ 8.53 (t, *J* = 5.7 Hz, 1H, Gly NH), 8.48 (d, *J* = 8.6 Hz, 1H, Cys NH), 6.38 (s, 1H, Urea NH), 5.60 (s, 2H, Urea NH_2), 4.44 (ddd, *J* = 9.0, 8.6, 4.5 Hz, 1H, Cys αCH), 3.71 (d, *J* = 5.7 Hz, 2H, Gly αCH_2), 3.47 (t, *J* = 6.6 Hz, 1H, Glu αCH), 3.02-2.98 (m, 2H, S$\underline{C}H_2CH_2CH_2NH$), 2.87 (dd, *J* =

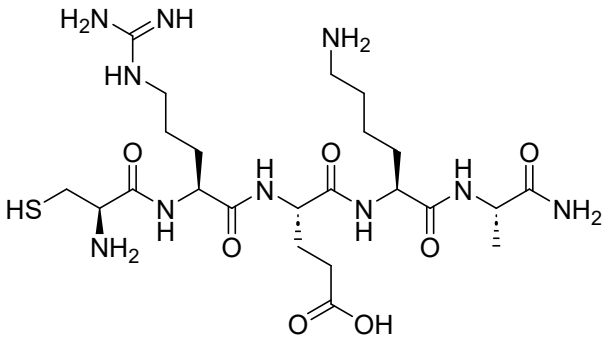
13.8, 4.4 Hz, 1H, Cys βCH$_2$), 2.64 (dd, *J* = 13.7, 9.0 Hz, 1H, Cys βCH$_2$), 2.53 (m, 2H, SCH$_2$C<u>H$_2$</u>CH$_2$NH), 2.34 (t, *J* = 7.3 Hz, 2H, Glu γCH$_2$), 1.92 (app. q, *J* = 7.3 Hz, 2H, Glu βCH), 1.62-1.54 (m, 2H, SCH$_2$CH$_2$C<u>H$_2$</u>NH) ppm.**^{13}C NMR** (101 MHz, DMSO-d_6): δ 172.3 (C=O), 171.6 (C=O), 171.5 (C=O), 171.3 (C=O), 159.5 (C=O), 53.5 (Glu αCH), 53.3 (Cys αCH), 41.7 (Gly αCH$_2$), 38.7 (S<u>C</u>H$_2$CH$_2$CH$_2$NH), 34.1 (Cys βCH$_2$), 32.0 (Glu γCH), 30.4 (SCH$_2$CH$_2$<u>C</u>H$_2$NH), 29.6 (Glu βCH$_2$), 27.3 (SCH$_2$<u>C</u>H$_2$CH$_2$NH) ppm. **HRMS** (*m/z* ESI$^+$): found 408.155190 ([M+H]$^+$, $C_{14}H_{26}N_5O_7S$ requires 408.1547). **ν$_{max}$** (film)/cm^{-1}: 3280 (N-H), 1644 (C=O).

_N_-((_R_)-3-(((_R_)-3-amino-3-carboxypropyl)thio)-1-((carboxymethyl)amino)-1-oxopropan-2-yl)-D-glutamine (20).

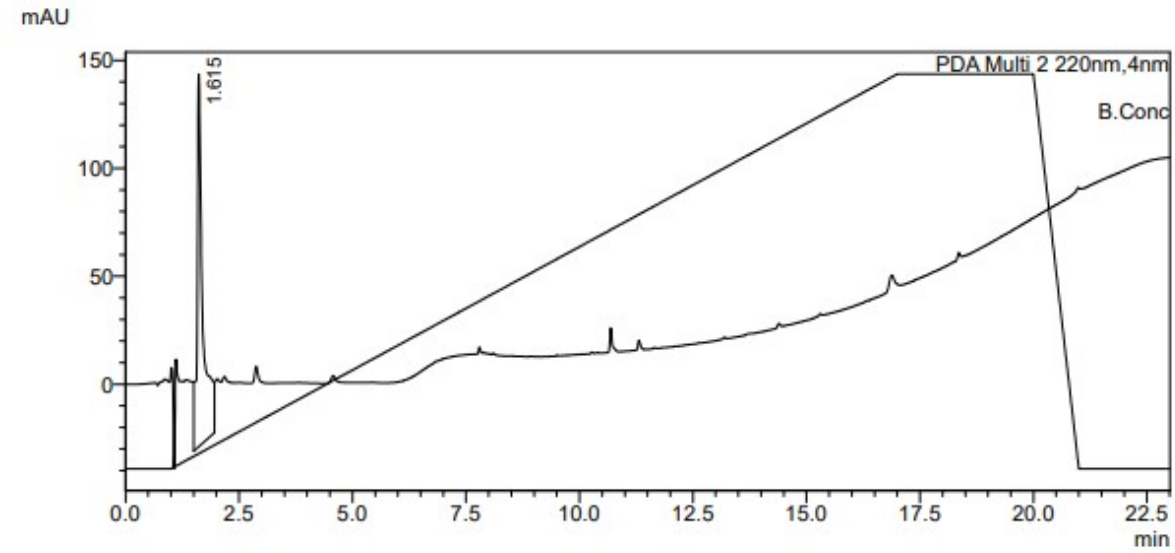


20 was prepared by radical mediated thiol-ene reaction of glutathione (**1**) and vinylglycine **12** as per **general procedure A** in batch (50%) and as per **general procedure B** under continuous flow (63%). The reaction mixture was washed with CH_2Cl_2 (2 x 50 mL) and aqueous layer was lyophilized prior to ^{1}H NMR spectroscopic analysis. **m.p.** 210-240 °C (dec.). **R$_f$** = 0.95 (H_2O:ACN, 7:3) reverse phase C_{18} TLC.**^{1}H NMR** (400 MHz, D_2O): δ 4.62 (dd, *J* = 8.4, 5.3 Hz, 1H, Cys *α*CH), 4.03 (s, 2H, Gly *α*CH$_2$), 4.03 (d, *J* = 12.6 Hz, 1H, SCH$_2$CH$_2$C<u>H</u>), 3.95-3.91 (t, *J* =6.3 Hz, 1H, Glu *α*CH), 3.11 (dd, *J* = 14.0, 5.3 Hz, 1H, Cys *β*CH$_2$), 2.94 (dt, *J* = 14.0, 8.4 Hz, 1H, Cys *β*CH$_2$), 2.75 (t, *J* = 7.5 Hz, 2H, Glu *γ*CH$_2$), 2.63-2.54 (m, 2H, Glu *β*CH$_2$), 2.24-2.18 (m, 4H, SCH$_2$C<u>H$_2$</u>CH+ Glu βCH$_2$) ppm. **^{13}C NMR** (101 MHz, D_2O): δ 174.7 (C=O), 173.1 (C=O), 173.0 (C=O), 173.0 (C=O), 172.7 (C=O), 53.3 (Glu *α*CH), 53.0 (Cys *α*CH), 52.8 (SCH$_2$CH$_2$<u>C</u>H), 41.2 (Gly *α*CH), 32.5 (Cys *β*CH$_2$), 31.1 (Glu *β*CH$_2$), 29.9 (S<u>C</u>H$_2$CH$_2$CH), 27.0 (Glu *γ*CH$_2$), 25.8 (SCH$_2$<u>C</u>H$_2$CH) ppm. **HRMS** (*m/z* ESI$^+$): found 409.1389 ([M+H]$^+$, $C_{14}H_{25}N_4O_8S$ requires 409.1387). **ν$_{max}$** (film)/cm^{-1}: 2924 (CH$_2$), 1725 (C=O), 1635 (C=O), 1217 (C-O).

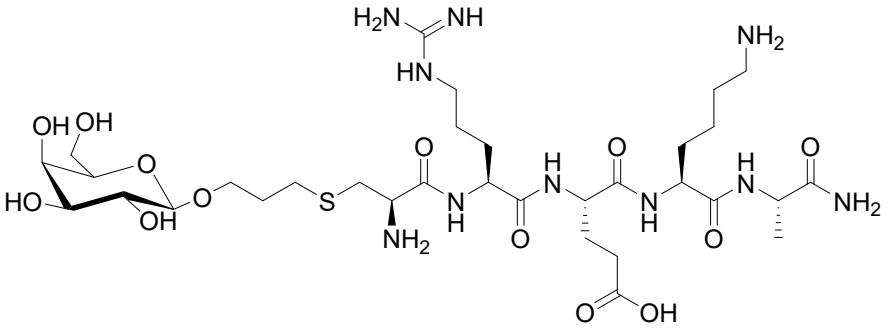
(*S*)-5-(((*S*)-6-amino-1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)amino)-4-((*S*)-2-((*R*)-2-amino-3-mercaptopropanamido)-5-guanidinopentanamido)-5-oxopentanoic acid (22)



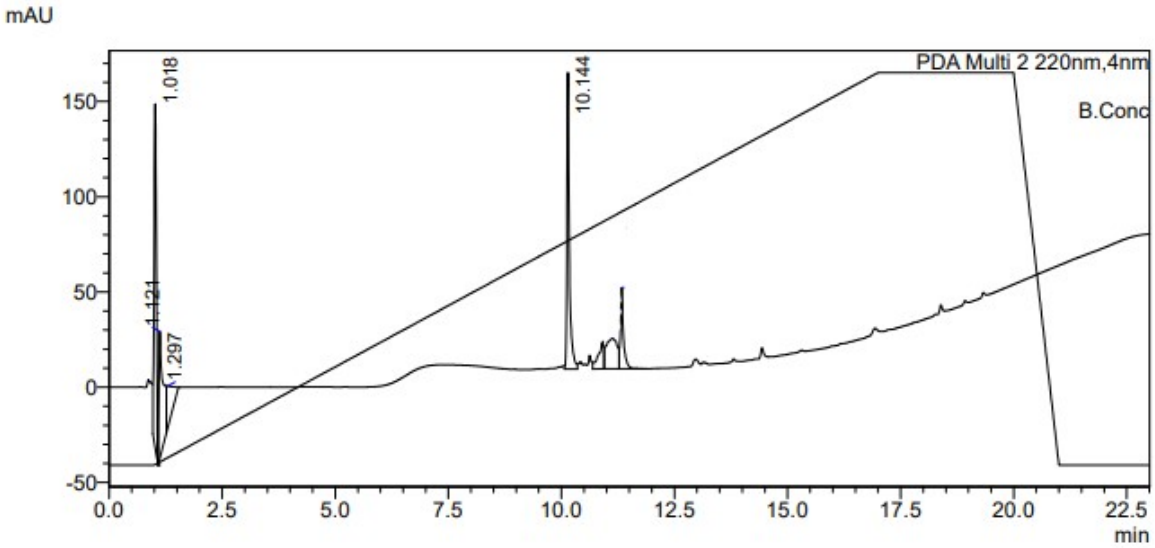
Peptide **22** was prepared as per **general procedure E** utilising Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Glu(O^{t}Bu)-OH, Fmoc-Arg(Pbf) and Fmoc-Cys(Trt)-OH. The crude peptide was purified by semi-preparatory RP-HPLC (C_{18} Φ4.6 × 250 mm column, 1 mL min^{-1} flow rate). **m.p.** 202-212 °C (dec.). **Retention time:** 1.62 min (5 - 95% ACN, 20 min 0.1% TFA, λ = 214 nm). **HRMS** (*m/z* ESI$^+$): found 605.3174 ([M+H]$^+$, $C_{23}H_{45}N_{10}O_7S$ requires 605.3188). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3305 (O-H, N-H), 1667 (C=O), 1202 (C-N).



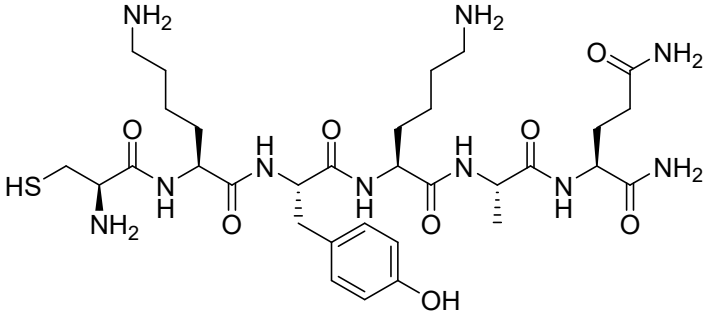
(*S*)-5-(((*S*)-6-amino-1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)amino)-4-((*S*)-2-((*R*)-2-amino-3-((3-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)propyl)thio)propanamido)-5-guanidinopentanamido)-5-oxopentanoic acid (23)



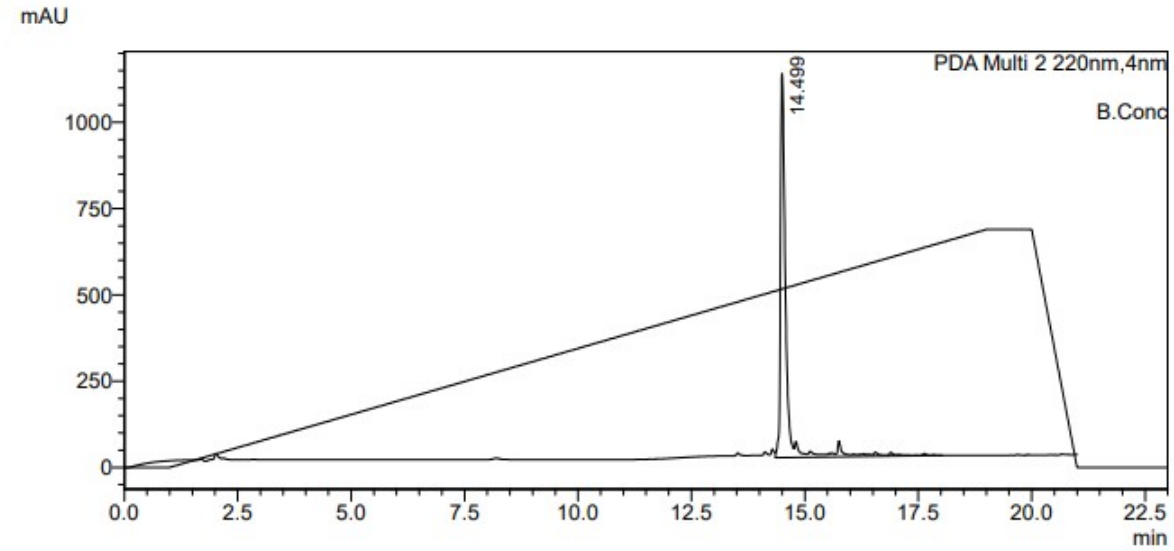
A solution of CREKA Peptide **22** (10 mg, 16.5 µmol), alkene (72.8 mg, 0.33 mmol) and photoinitiator (9 mg, 41.3 µmol) in H_2O in 0.1% formic acid (2 mL) was pumped at 500 µL min^{-1} with 20 mm syringe diameter through the coil reactor at room temperature under UV irradiation (352 nm, 110 V). Residence time was 120 min. Solvent was removed *in vacuo*. Glycosylated peptide **23** was purified by analytical RP-HPLC (C_{18}, 100 Å x 4.6 mm, 5 µm LC column). Glycosylated peptide **23** was afforded in 44% as isolated yield. **Retention time:** 10.14 min (5 - 95% MeOH, 20 min 0.1% TFA, λ = 220 nm). **HRMS** (*m/z* ESI^+): found 825.4111 ($[M+H]^+$, $C_{32}H_{61}N_{10}O_{13}S$ requires 825.4135). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3325 (O-H, N-H), 1657 (C=O), 1050 (C-O).



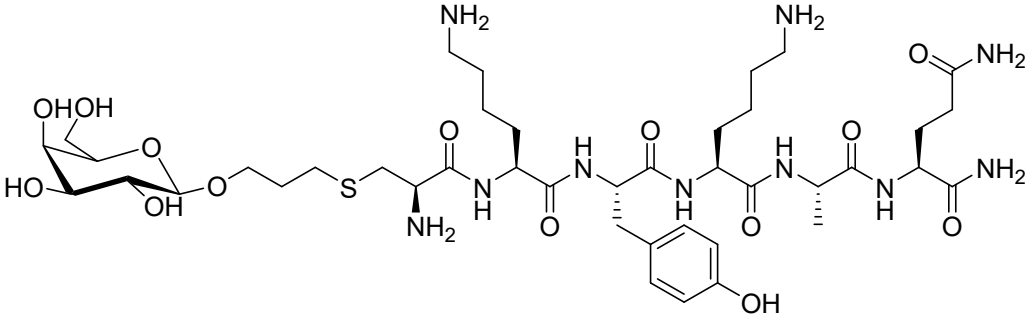
(S)-2-((2S,5S,8S,11S,14R)-14-amino-5,11-bis(4-aminobutyl)-8-(4-hydroxybenzyl)-15-mercapto-2-methyl-4,7,10,13-tetraoxo-3,6,9,12-tetraazapentadecanamido)pentanediamide (24)



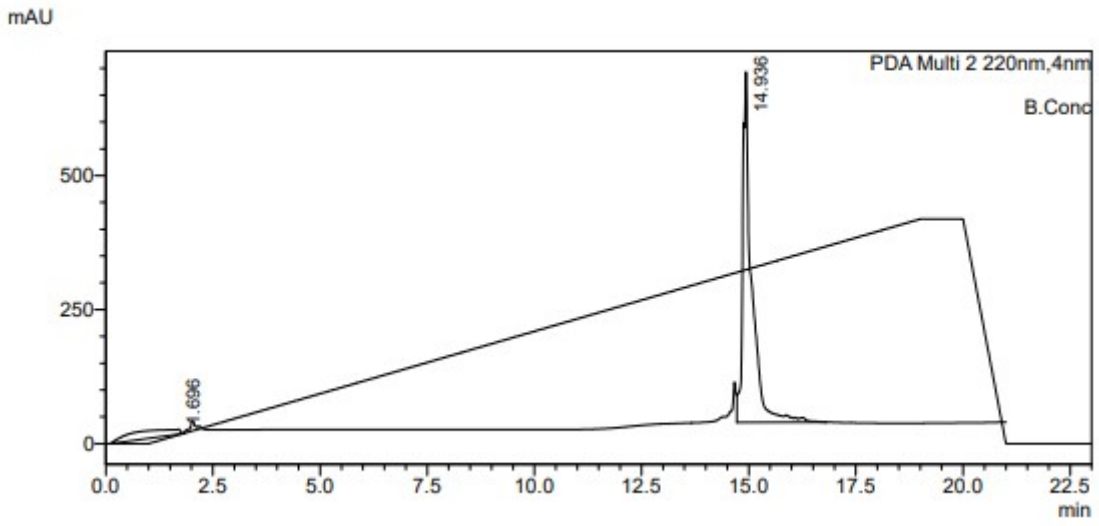
AFP peptide **24** was prepared as per **general procedure E** utilising Fmoc-Gln(trt)-OH, Fmoc-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH, (x 2), Lys(Boc)-OH and Fmoc-Cys(Trt)-OH. The crude peptide was purified by semi-preparatory RP-HPLC (C_{18} Φ4.6 × 250 mm column, 1 mL min^{-1} flow rate). **m.p**: 220-224 °C (dec.). **Retention time:** 14.50 min (2 - 60% ACN, 20 min 0.1% TFA, λ = 254 nm). **HRMS** (*m/z* ESI^+): found 739.3925 ($[M+H]^+$, $C_{32}H_{55}N_{10}O_8S$ requires 739.3920). ν_{max} (film)/cm^{-1}: 3280 (O-H, N-H), 1675 (C=O), 1625 (C=C), 1457 (C-H), 1202 (C-N), 1138 (C-N), 1024 (C-O), 694 (C=C).



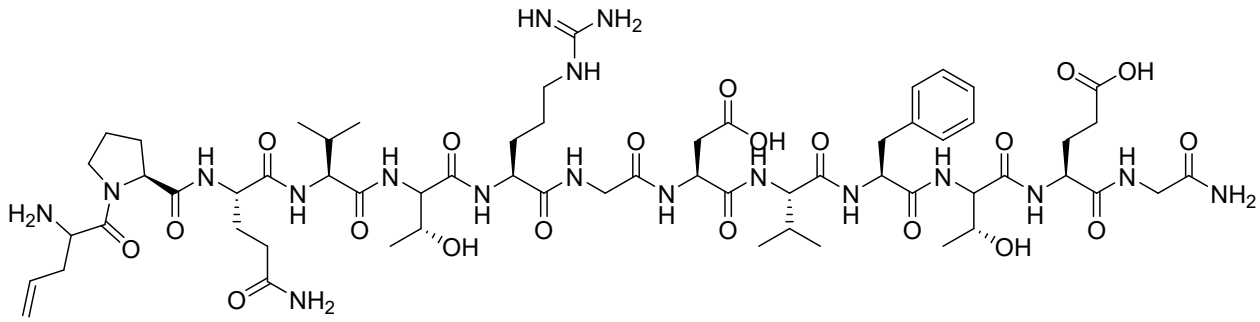
(S)-2-((2S,5S,8S,11S,14R)-14-amino-5,11-bis(4-aminobutyl)-8-(4-hydroxybenzyl)-2-methyl-4,7,10,13-tetraoxo-19-(((2R,3R,4S,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-16-thia-3,6,9,12-tetraazanonadecanamido)pentanediamide (25)



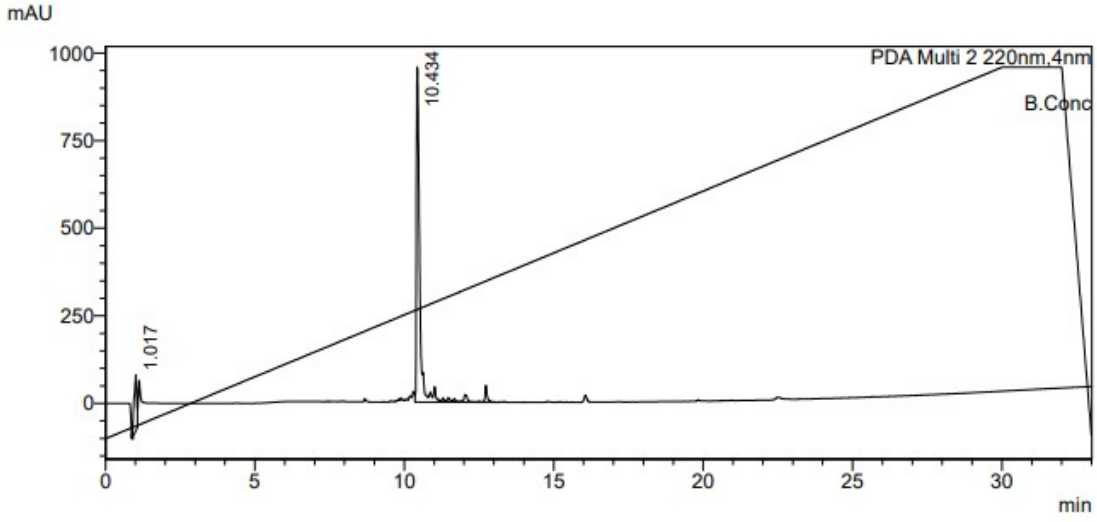
A solution of AFP Peptide **24** (10 mg, 13.5 µmol), alkene (0.27 mmol) and photoinitiator (2.5 eq., 33.8 µmol) in H_2O in 0.1% formic acid (2 mL) was pumped at 500 µL min^{-1} with 20 mm syringe diameter through the coil reactor at room temperature under UV irradiation (352 nm, 110 V). Residence time was 120 min. Solvent was removed *in vacuo*. Glycosylated peptide **25** was purified by analytical RP-HPLC (C_{18}, 100 Å x 4.6 mm, 5 µm LC column). Glycosylated peptide **25** was afforded in 58% yield. **Retention time:** 14.94 min (5 - 95% ACN, 20 min 0.1% TFA, λ = 220 nm). **HRMS** (*m/z* ESI^+): found 480.2477 ($[M+2H]^{2+}$, $C_{41}H_{72}N_{10}O_{14}S$ requires 480.2470). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3354 (O-H), 1672(C=O), 1033 (C-O).



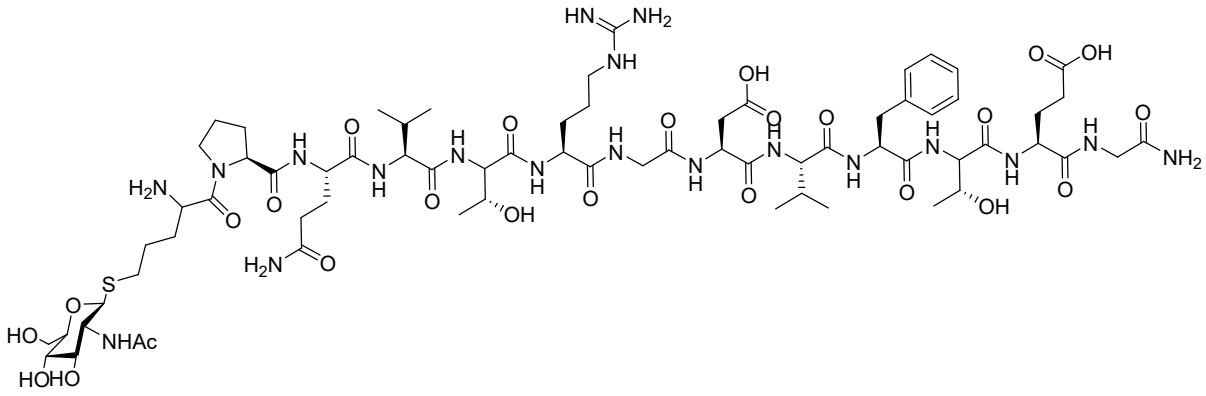
(3S,6S,9S,12S,15S)-15-((2-amino-2-oxoethyl)carbamoyl)-3-((3S,6S,9S,12S)-3-(3-amino-3-oxopropyl)-1-((2S)-1-(2-aminopent-4-enoyl)pyrrolidin-2-yl)-12-(3-guanidinopropyl)-9-((R)-1-hydroxyethyl)-6-isopropyl-1,4,7,10,13-pentaoxo-2,5,8,11,14-pentaazahexadecan-16-amido)-9-benzyl-12-((R)-1-hydroxyethyl)-6-isopropyl-4,7,10,13-tetraoxo-5,8,11,14-tetraazaoctadecanedioic acid (26)



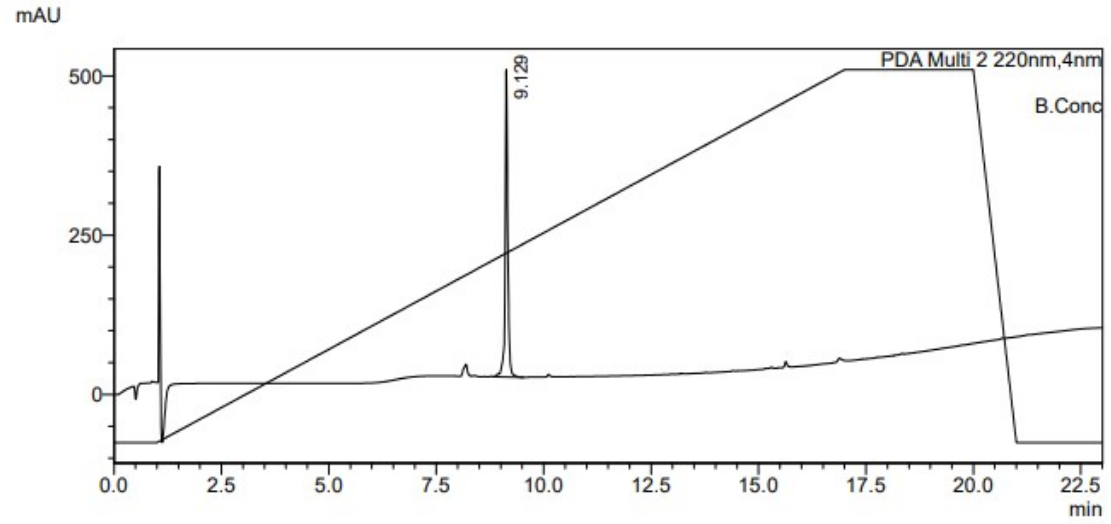
RGD peptide **26** was prepared by Liberty Blue® peptide synthetiser utilising Fmoc-Gly-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Val-OH, Fmoc-Gln(trt)-OH, Fmoc-Pro-OH. Last amino acid was coupled as per **general procedure E** utilising Fmoc-allyl-Gly-OH. The crude peptide was purified by semi-preparatory RP-HPLC (C_{18} Φ4.6 × 250 mm column, 1 mL min^{-1} flow rate). **m.p.** 237-241°C (dec.). **Retention time:** 10.43 min (5 - 95% ACN, 30 min 0.1% TFA, λ = 220 nm). **HRMS** (*m/z* ESI^+): found 1401.7104 ($[M+H]^+$, $C_{61}H_{97}N_{18}O_{20}$ requires 1401.7121). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3280 (O-H, N-H), 1623 (C=O), 1022 (C-N), 698.56 (C=C).



(3S,6S,9S,12S,15S)-3-((3S,6S,9S,12S)-1-((2S)-1-(5-(((2S,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)thio)-2-aminopentanoyl)pyrrolidin-2-yl)-3-(3-amino-3-oxopropyl)-12-(3-guanidinopropyl)-9-((R)-1-hydroxyethyl)-6-isopropyl-1,4,7,10,13-pentaoxo-2,5,8,11,14-pentaazahexadecan-16-amido)-15-((2-amino-2-oxoethyl)carbamoyl)-9-benzyl-12-((R)-1-hydroxyethyl)-6-isopropyl-4,7,10,13-tetraoxo-5,8,11,14-tetraazaoctadecanedioic acid (28)



A solution of RGD Peptide **26** (10 mg, 3.57 µmol), thiol **27** (4.23 mg, 0.017 mmol) and photoinitiator **21** (2.5 eq., 8.93 µmol) in DES: H_2O (EG:H_2O) (3:2, 1.5 mL) was pumped at 500 µL min^{-1} with 20 mm syringe diameter through the coil reactor at room temperature under UV irradiation (352 nm, 110 V). Residence time was 120 min. Solvent was removed *in vacuo*. Glycosylated peptide **28** was diluted in H_2O (2 mL) and purified by analytical RP-HPLC (C_{18}, 100 Å x 4.6 mm, 5 µm LC column). Glycosylated peptide **28** was afforded in 62% as isolated yield. **Retention time:** 9.13 min (5 - 95% ACN, 20 min 0.1% TFA, λ = 220 nm). **HRMS** (*m/z* ESI^+): found 819.8945 ($[M+2H]^{2+}$, $C_{69}H_{113}N_{19}O_{25}S$ requires 819.8932). $\boldsymbol{\nu_{max}}$ (film)/cm^{-1}: 3270 (O-H, N-H), 1625 (C=O), 1528 (C=C benzene), 1425 (O-H carboxylic) acid), 1200 (C-N), 1133 (C-O), 800 (C=C).



Analytical Calibrations

Table S1: Measures of retention times for different concentrations of GSH, standard deviation (s) and coefficient of variation (RSD) and RP-HPLC data. (2 - 15% ACN, 20 min 0.1% TFA, λ = 214 nm).

C ($mg \cdot ml^{-1}$)	Retention time (min)			Average	s	RSD (%)
1	1.911	1.916	1.903	1.91	0.00535	0.28
3.3	1.886	1.836	1.836	1.85	0.02357	1.27
4.8	1.873	1.879	1.875	1.88	0.00249	0.133
7.6	1.863	1.811	1.733	1.80	0.0534	2.96
9.7	1.853	1.754	1.789	1.80	0.0410	2.28

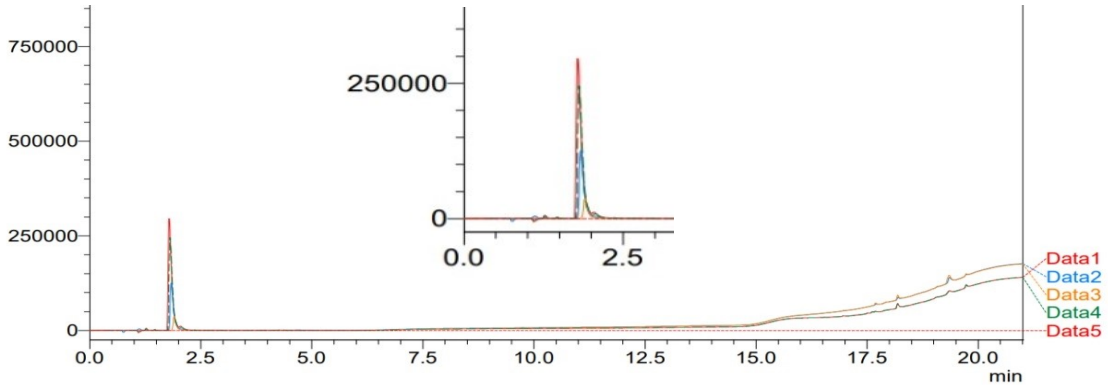


Figure **S1**: GSH **25** HPLC calibration data.

The suitability of the method was determined by quantifying the limit of detection (LOD) and limit of quantification (LOQ) of GSH calibration. LOD was evaluated by considering the analyte concentration that yield a signal-to-noise ratio (s/n) of 3. LOQ is the minimum concentration of the analyte that can be determined at an acceptable precision and accuracy under the analytical conditions used its calculated by determinate signal-to-noise ratio (s/n) of 10. LOD of this calibration was $5.08 \cdot 10^{-02}$ µmol mL^{-1} and LOQ 0.154 µmol mL^{-1}.

Table S2: Measures of different concentrations of GSH, peak area of GSH, and average between the 3 area measurements in RP-HPLC data. (2 - 15% ACN, 20 min 0.1% TFA, λ = 214 nm).

C ($mg \cdot ml^{-1}$)	Retention time (min)			Average	s	RSD (%)
1	1.911	1.916	1.903	1.91	0.00535	0.28
3.3	1.886	1.836	1.836	1.85	0.02357	1.27
4.8	1.873	1.879	1.875	1.88	0.00249	0.133
7.6	1.863	1.811	1.733	1.80	0.0534	2.96
9.7	1.853	1.754	1.789	1.80	0.0410	2.28

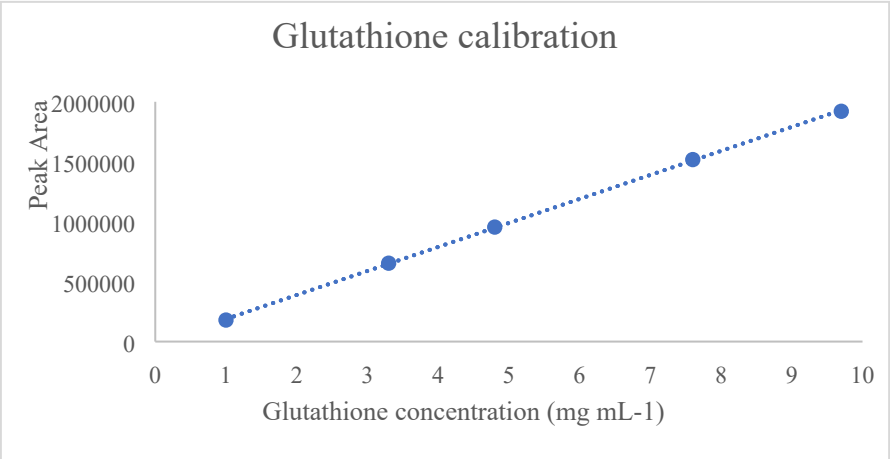
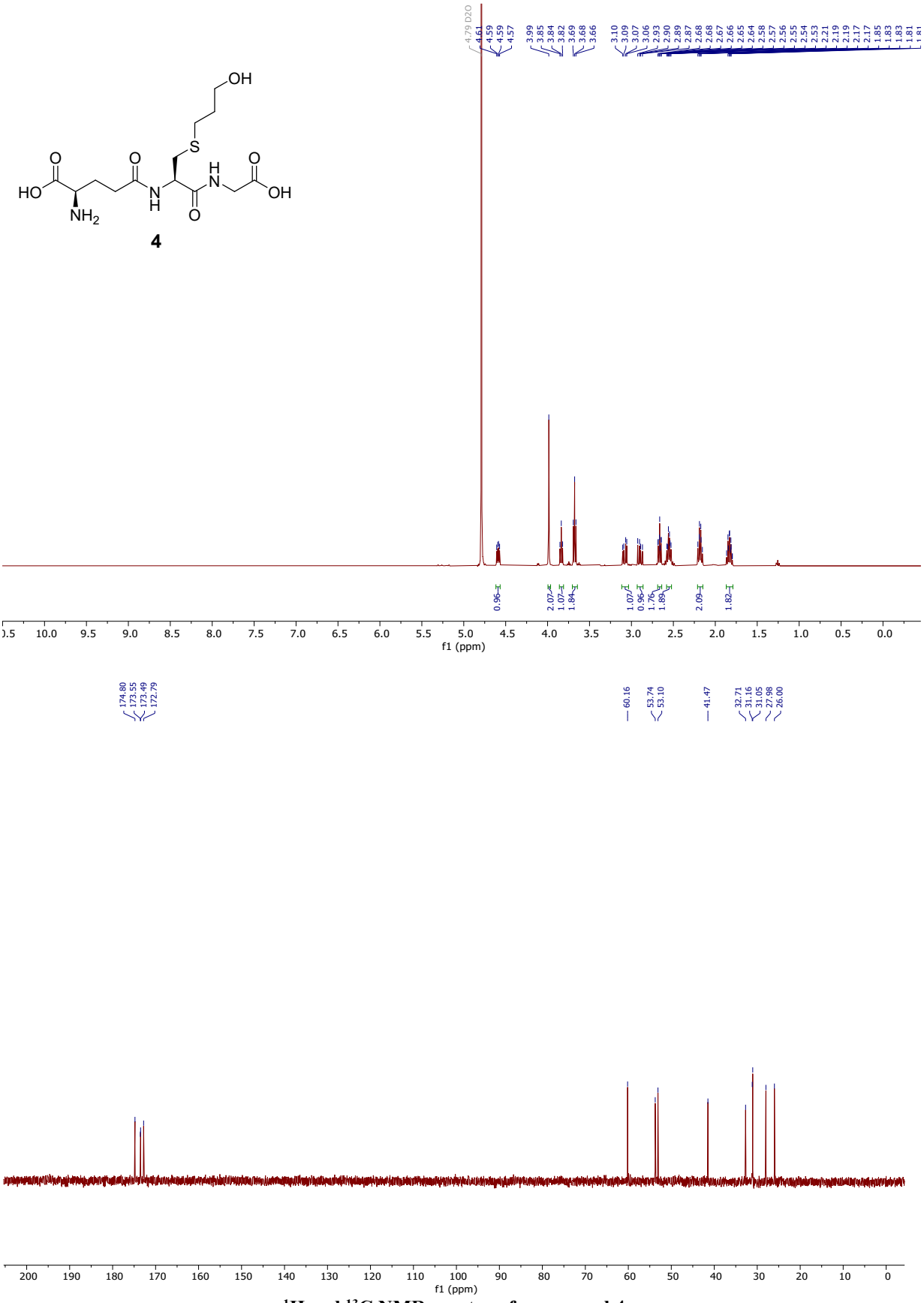
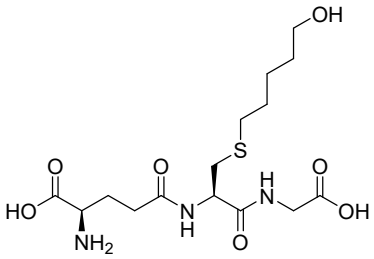


Figure **S1**: GSH **25** RP-HPLC calibration curve

NMR Spectra of Thiol-ene Products

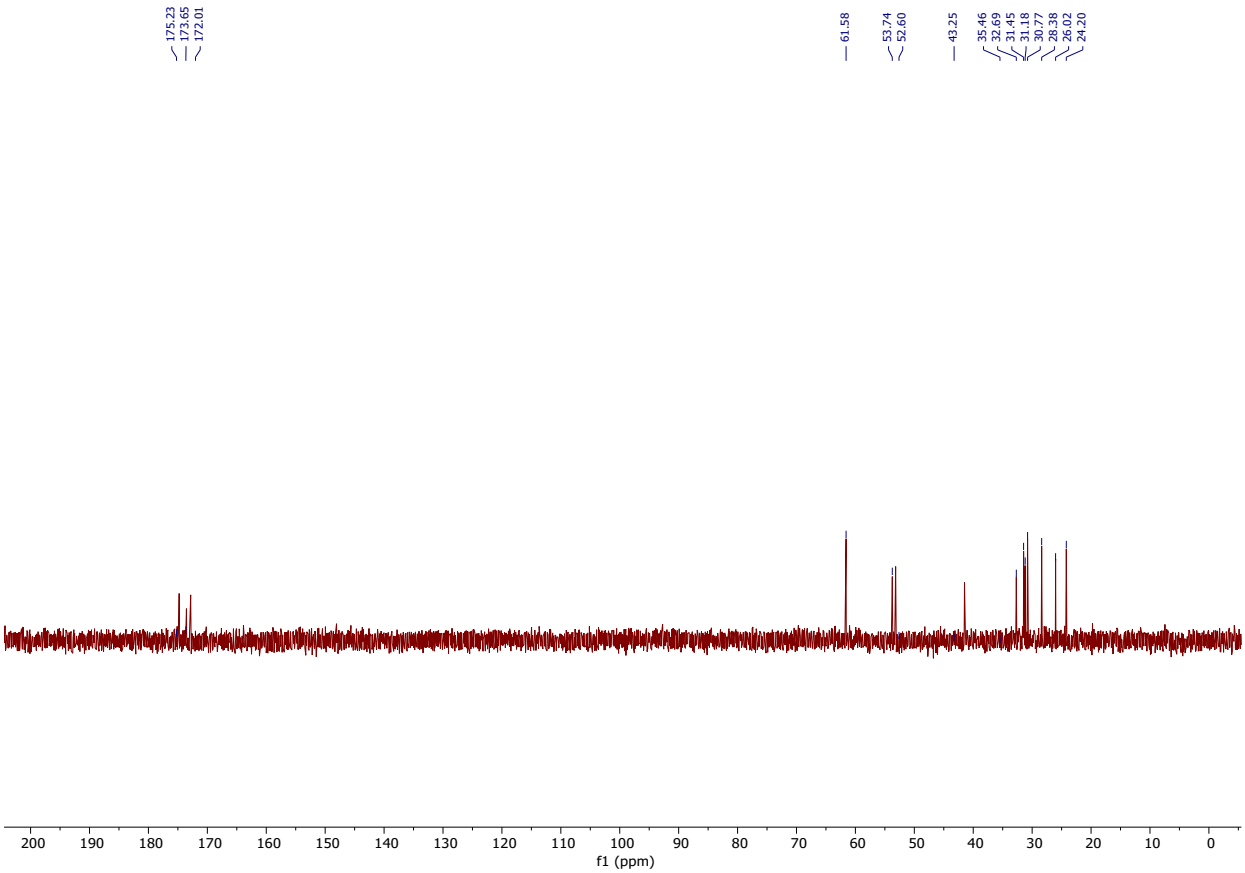


^{1}H and ^{13}C NMR spectra of compound 4

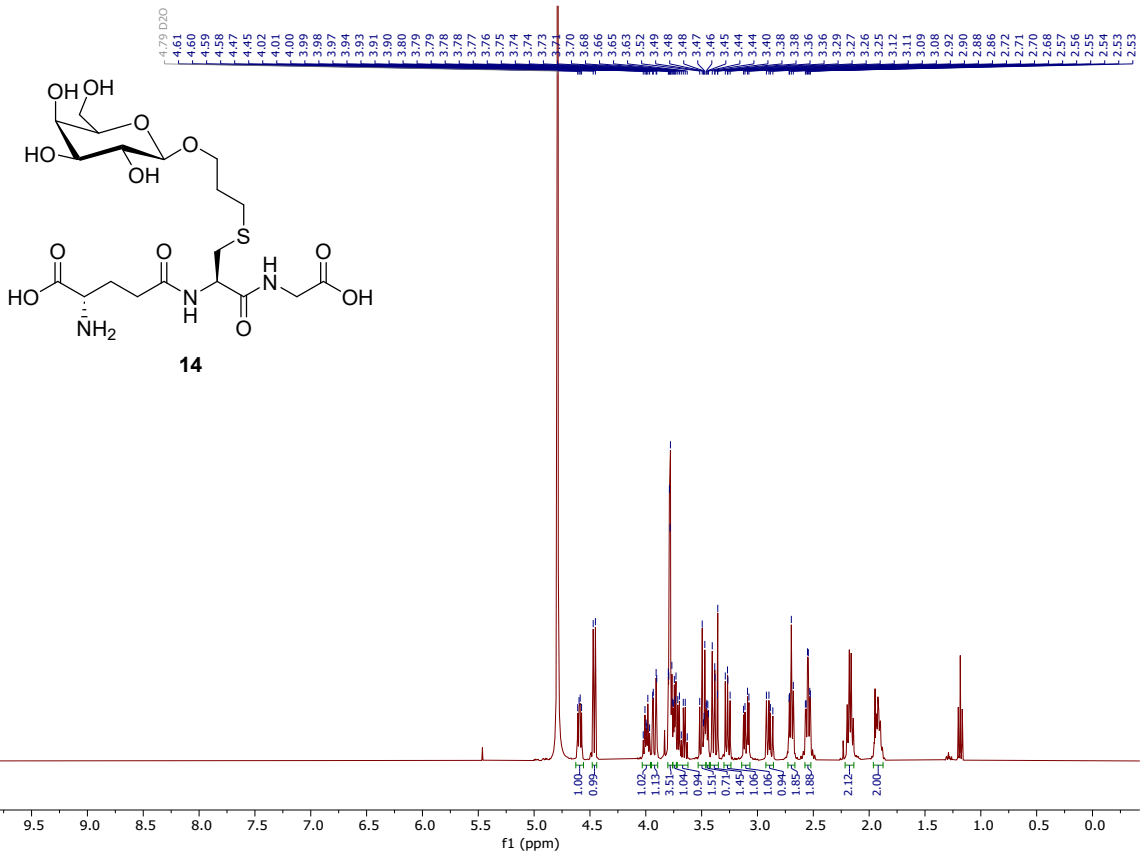

OH
S
O
O
HO
N
H
H
N
O
OH
NH2
O

13

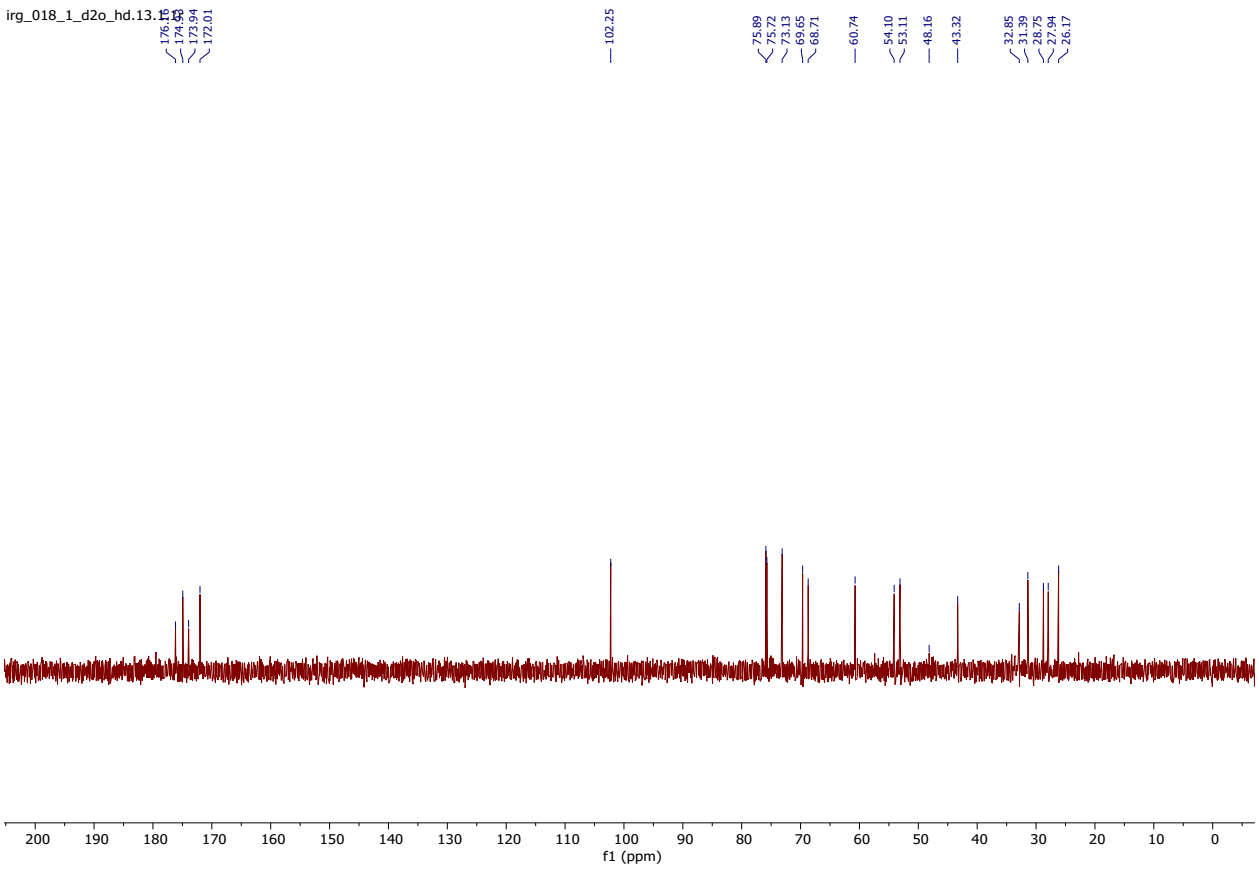
4.79 D2O
4.61
4.59
4.58
4.57
4.00
3.86
3.85
3.83
3.63
3.62
3.60
3.07
3.06
2.92
2.90
2.65
2.63
2.61
2.59
2.57
2.56
2.54
2.22
2.20
2.18
1.65
1.63
1.61
1.60
1.59
1.58
1.57
1.56
1.46
1.45
1.44
1.43
0.99
2.18
1.02
2.12
1.04
1.03
2.05
2.10
2.20
2.16
2.26
2.10
f1 (ppm)


175.23
173.65
172.01
61.58
53.74
52.60
43.25
35.46
32.69
31.45
31.18
30.77
28.38
26.02
24.20
f1 (ppm)

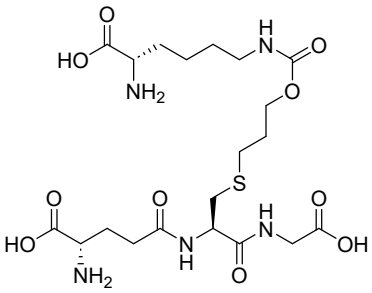
^{1}H and ^{13}C NMR spectra of compound 13



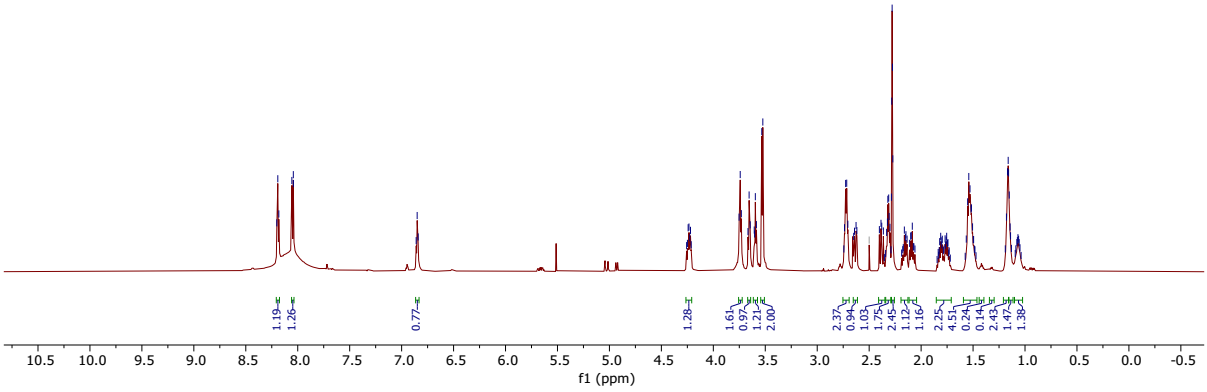
14

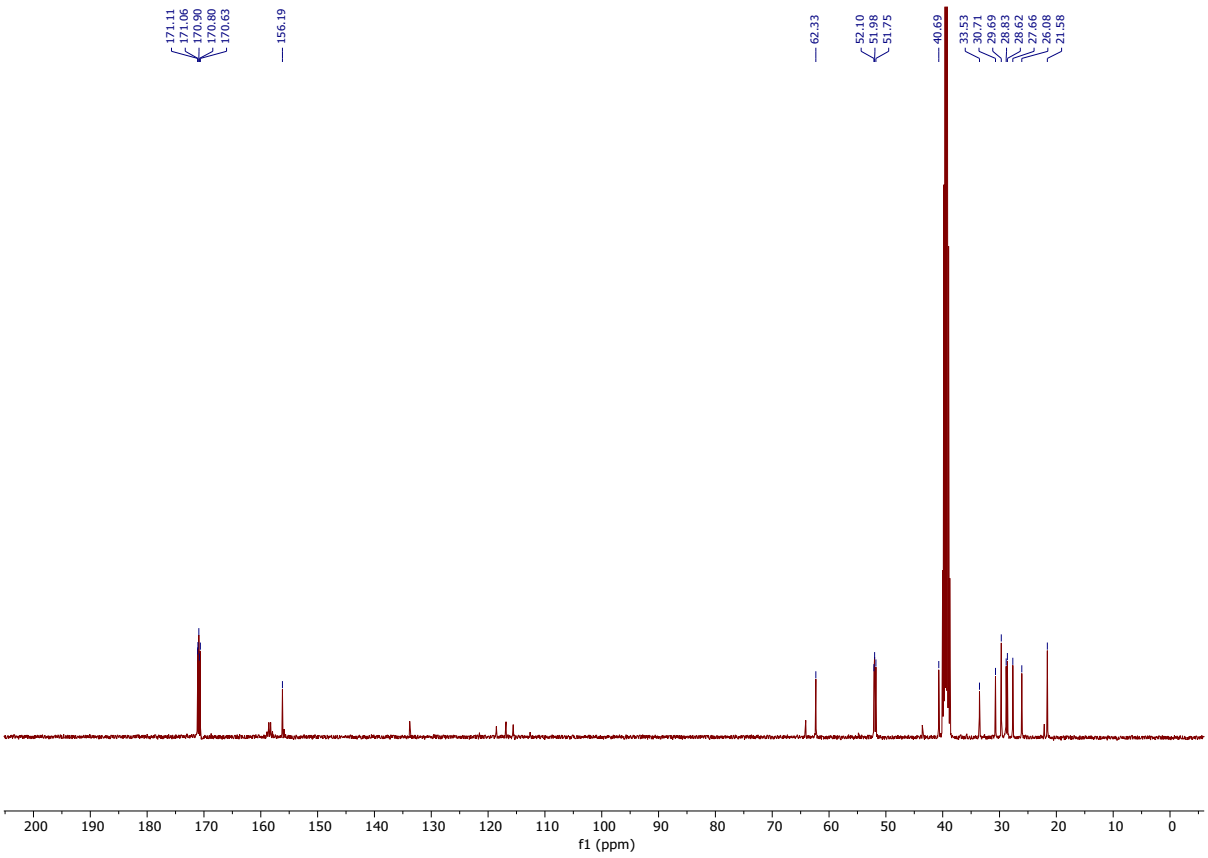


^{1}H and ^{13}C NMR spectra of compound 14

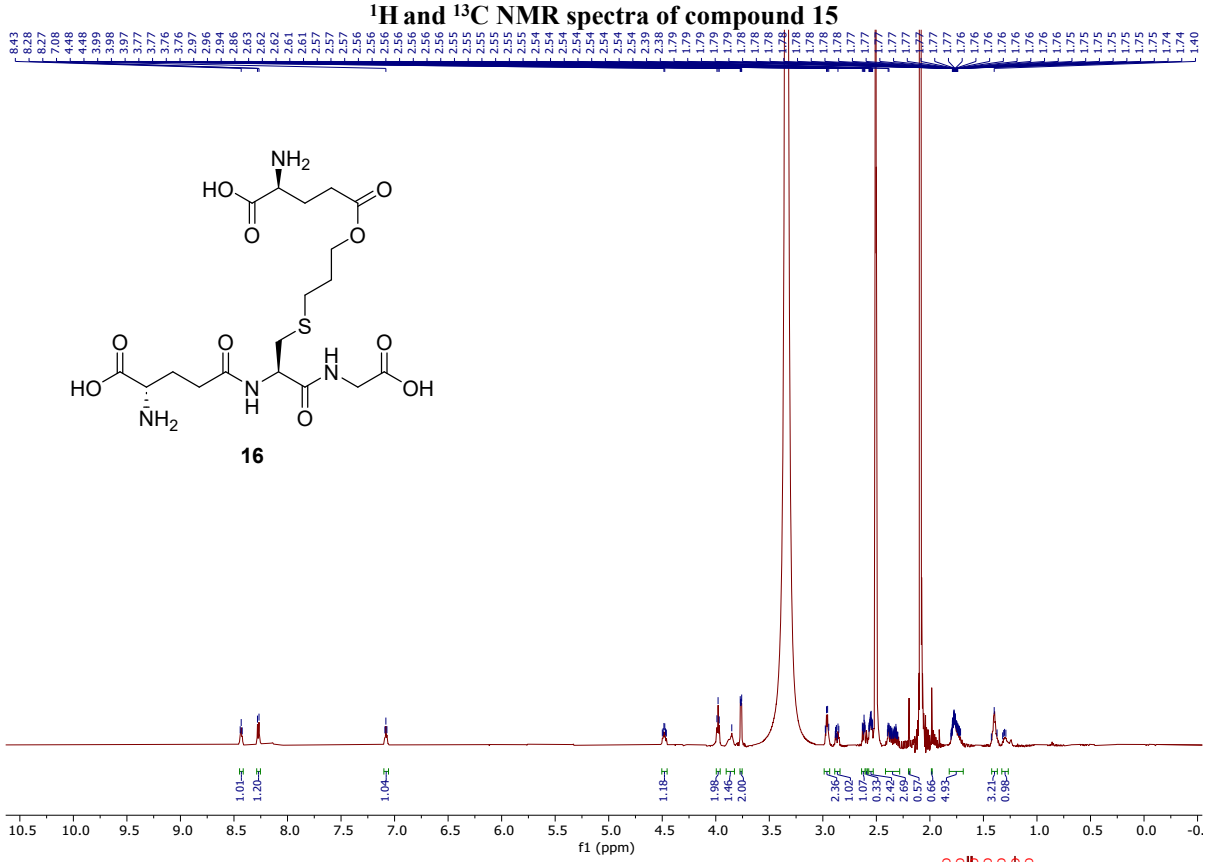


15

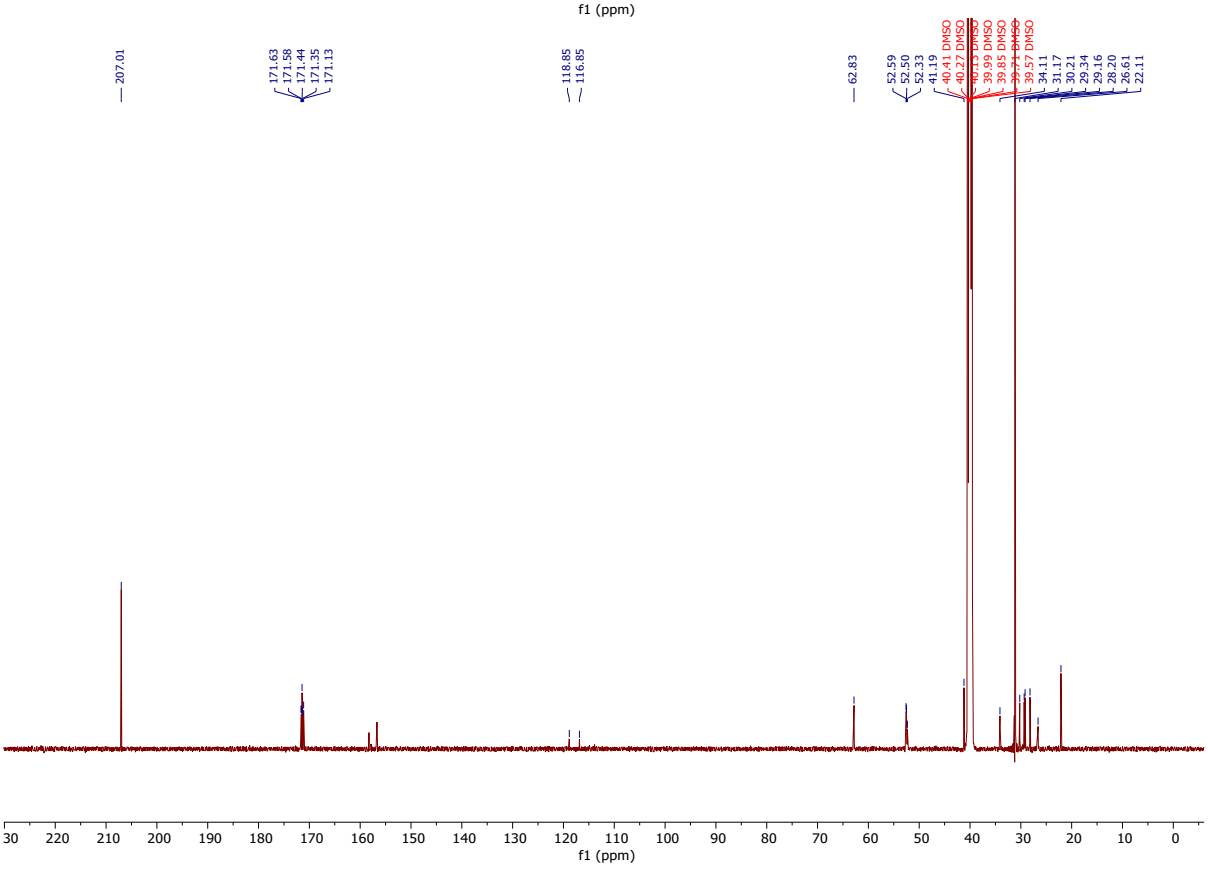




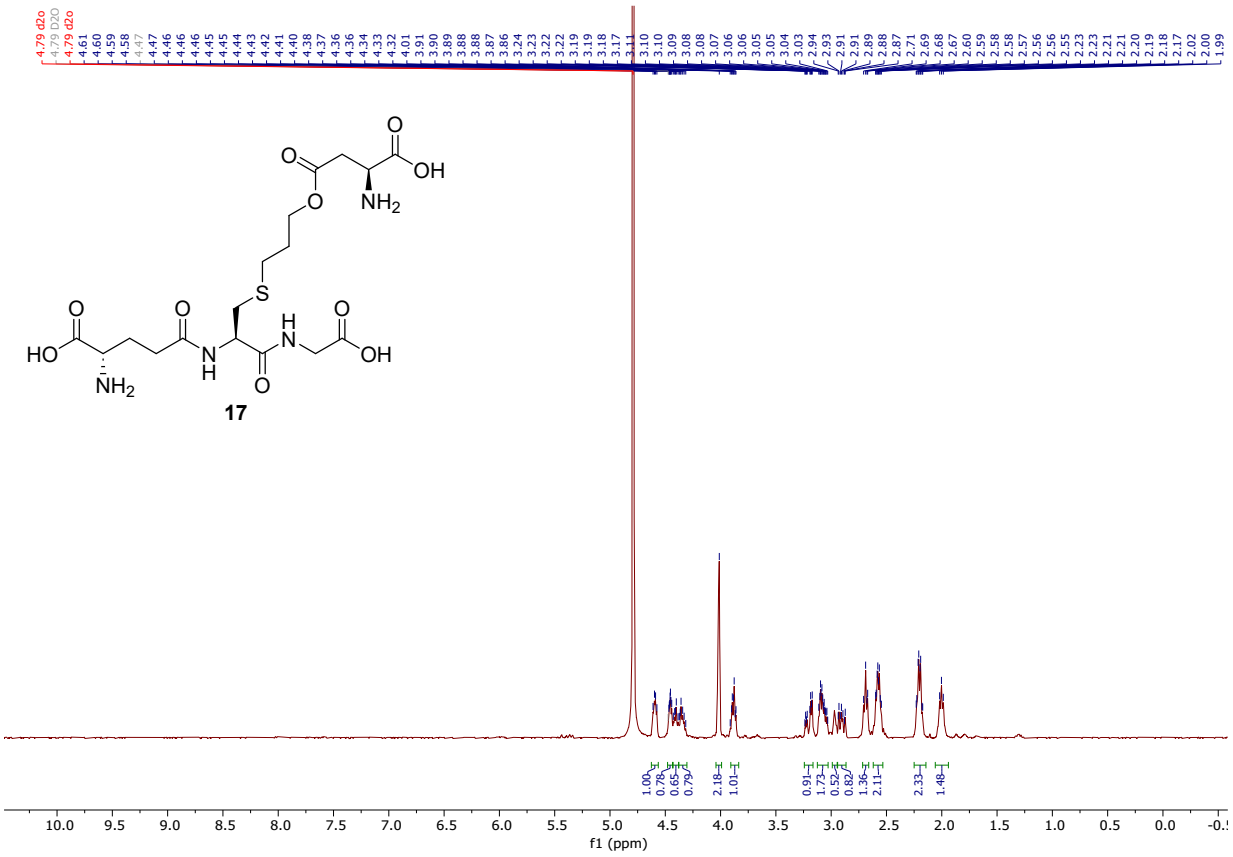
^{1}H and ^{13}C NMR spectra of compound 15

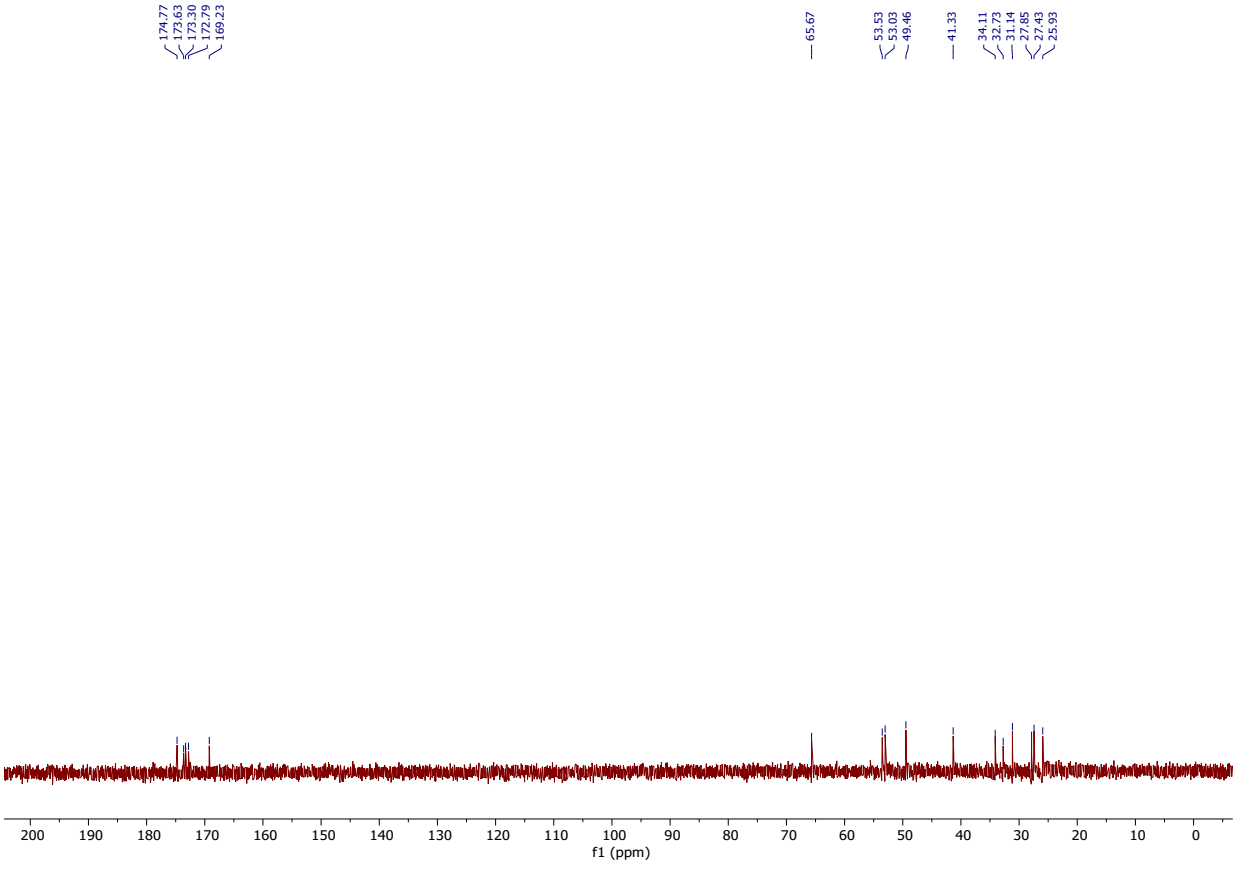


16

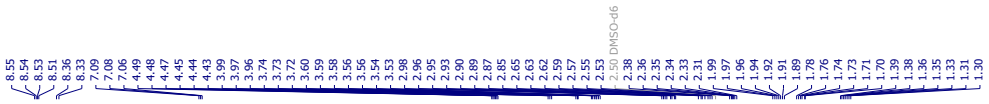


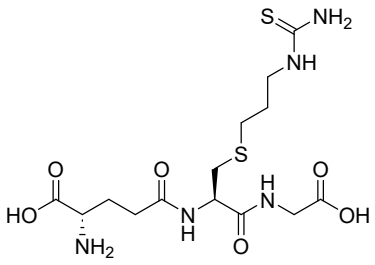
^{1}H and ^{13}C NMR spectra of compound 16



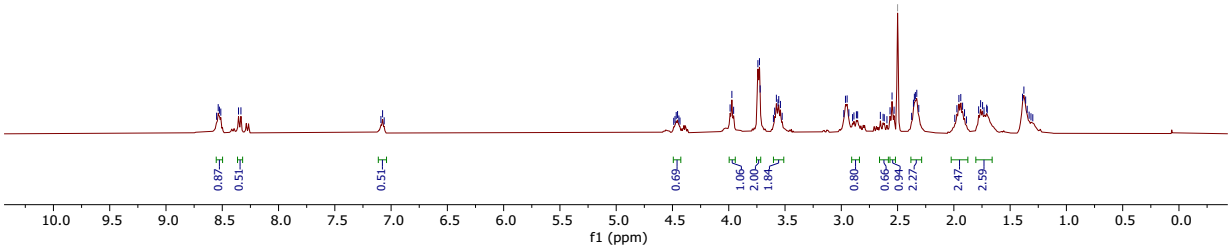


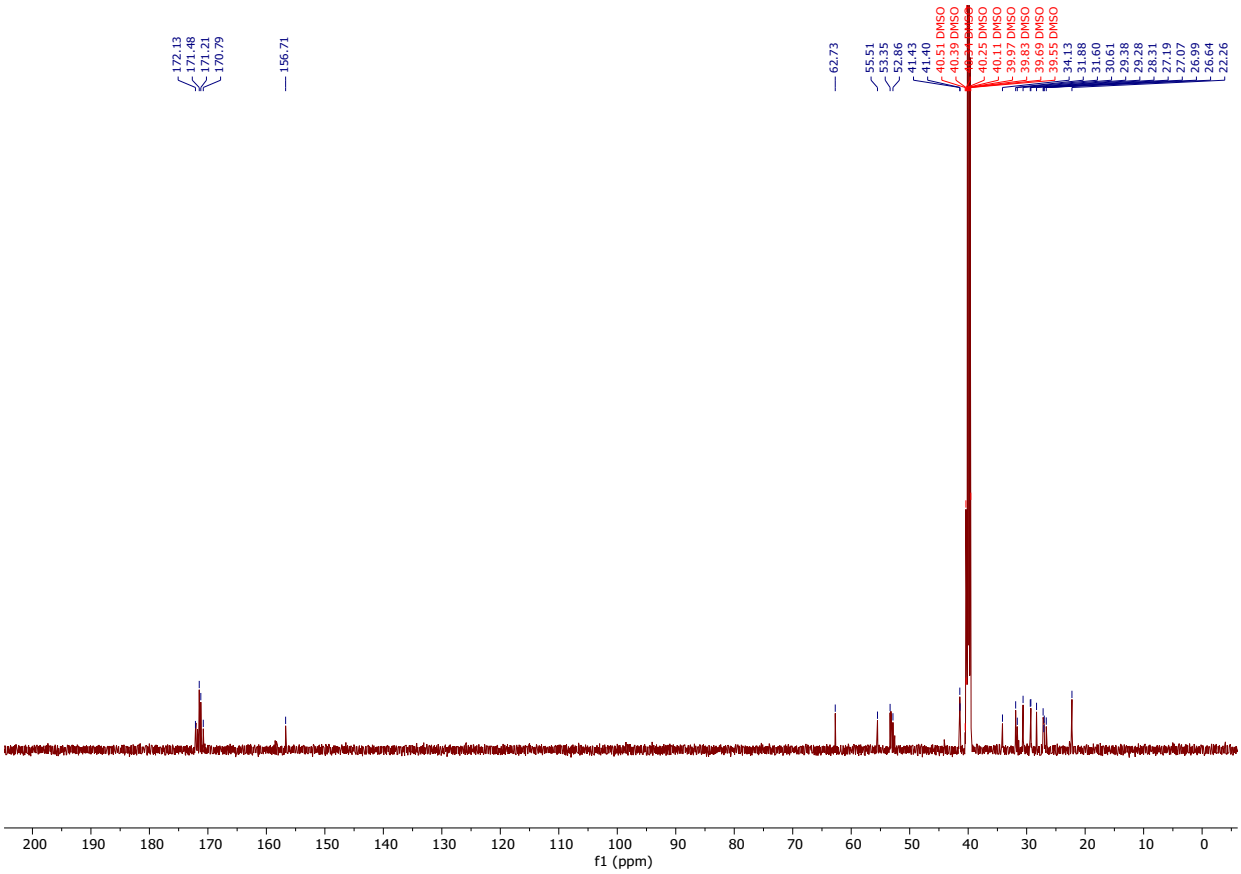
^{1}H and ^{13}C NMR spectra of compound 17



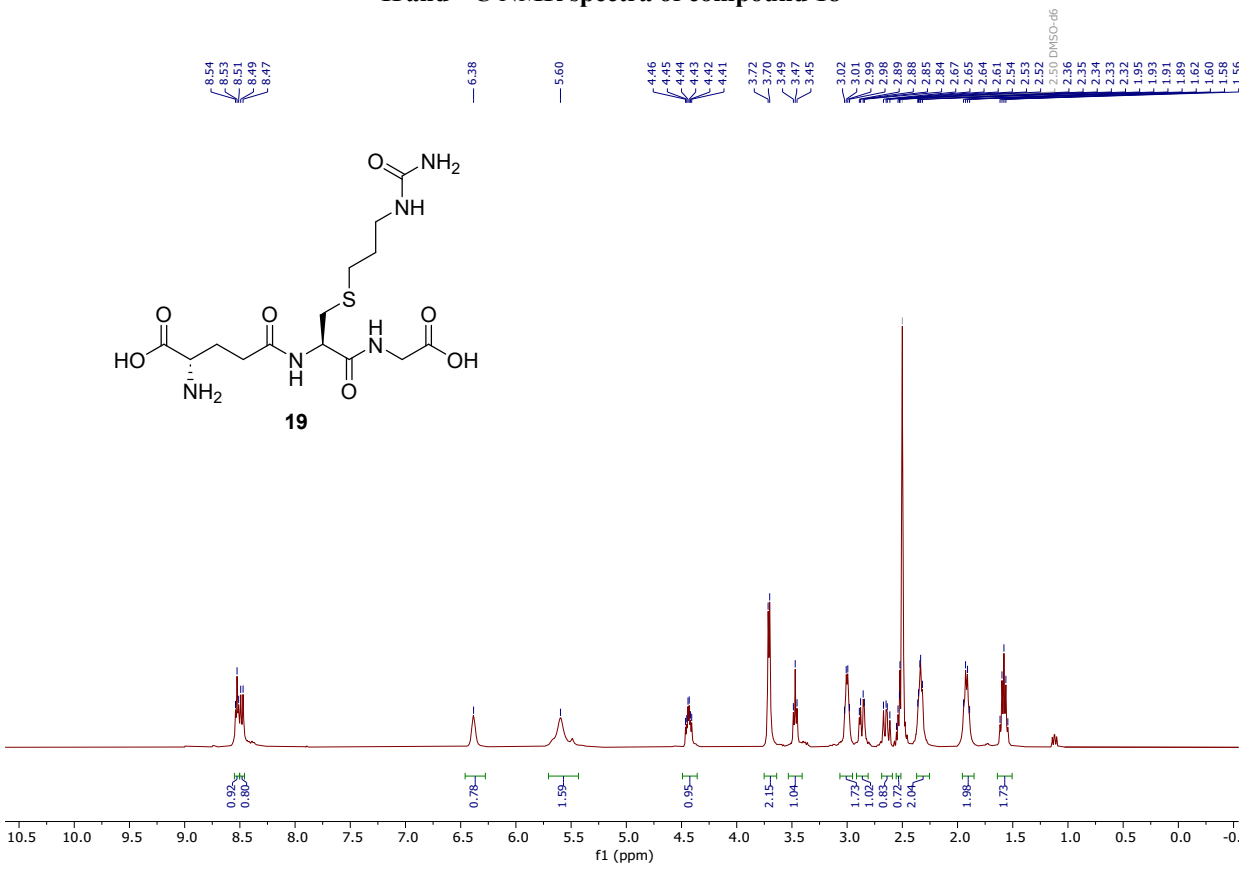


18

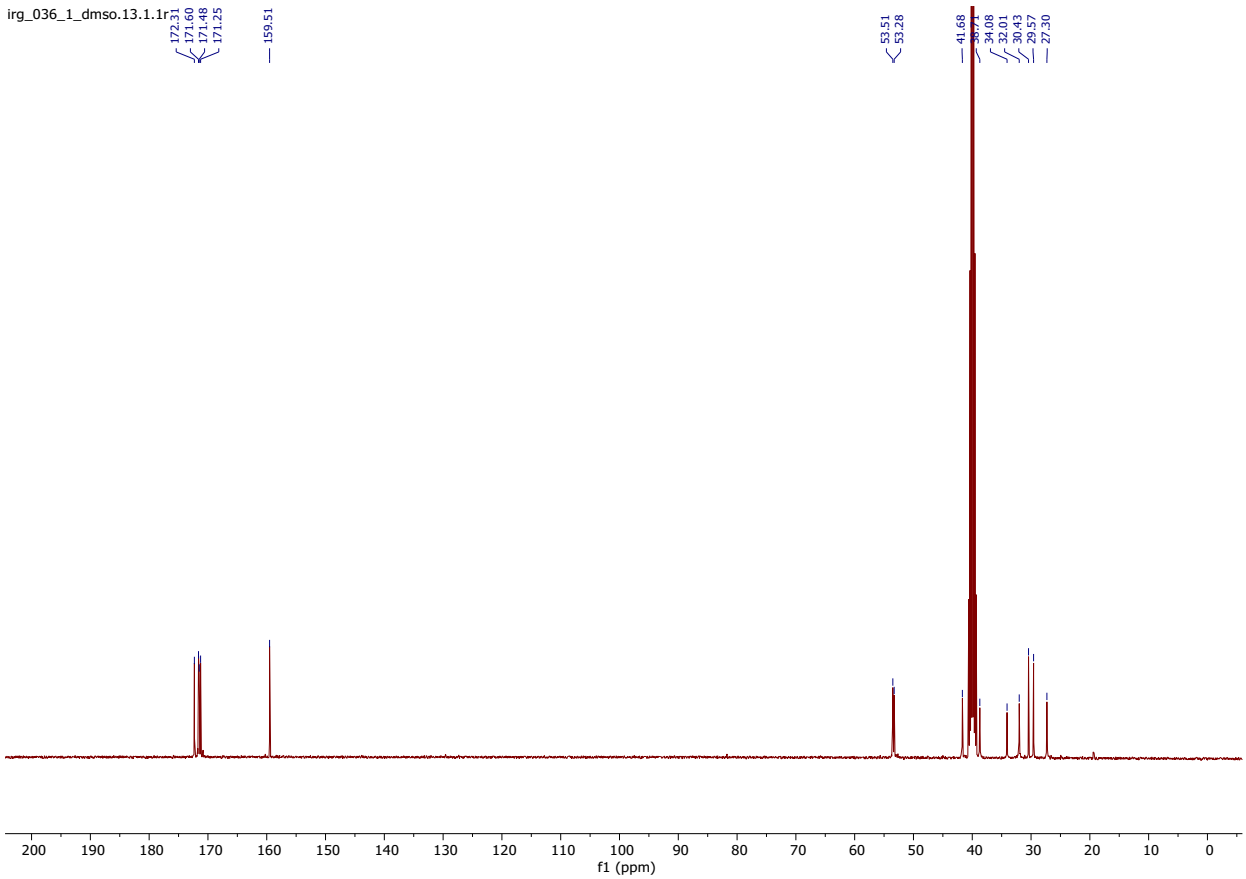




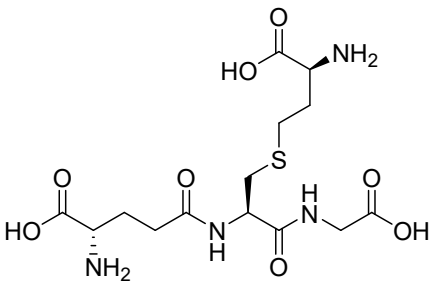
^{1}H and ^{13}C NMR spectra of compound 18



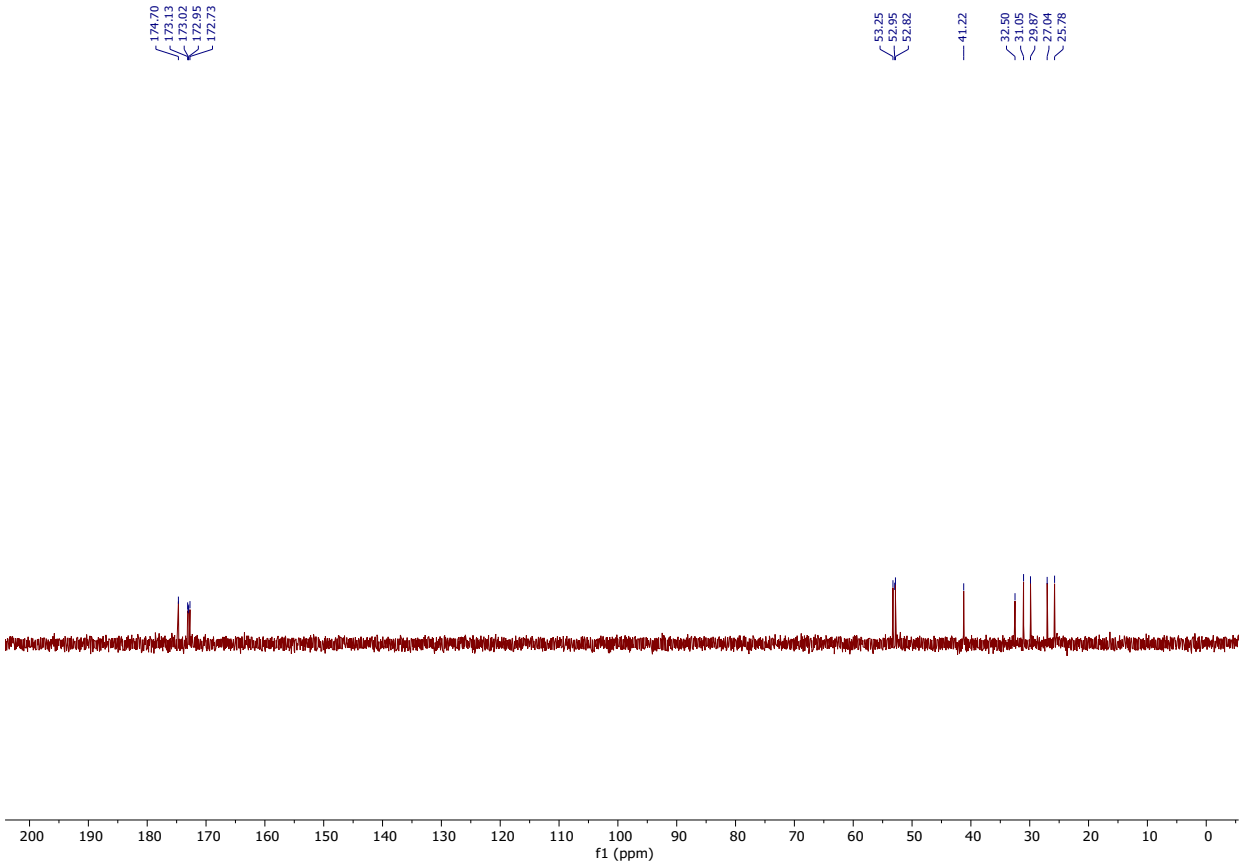
19



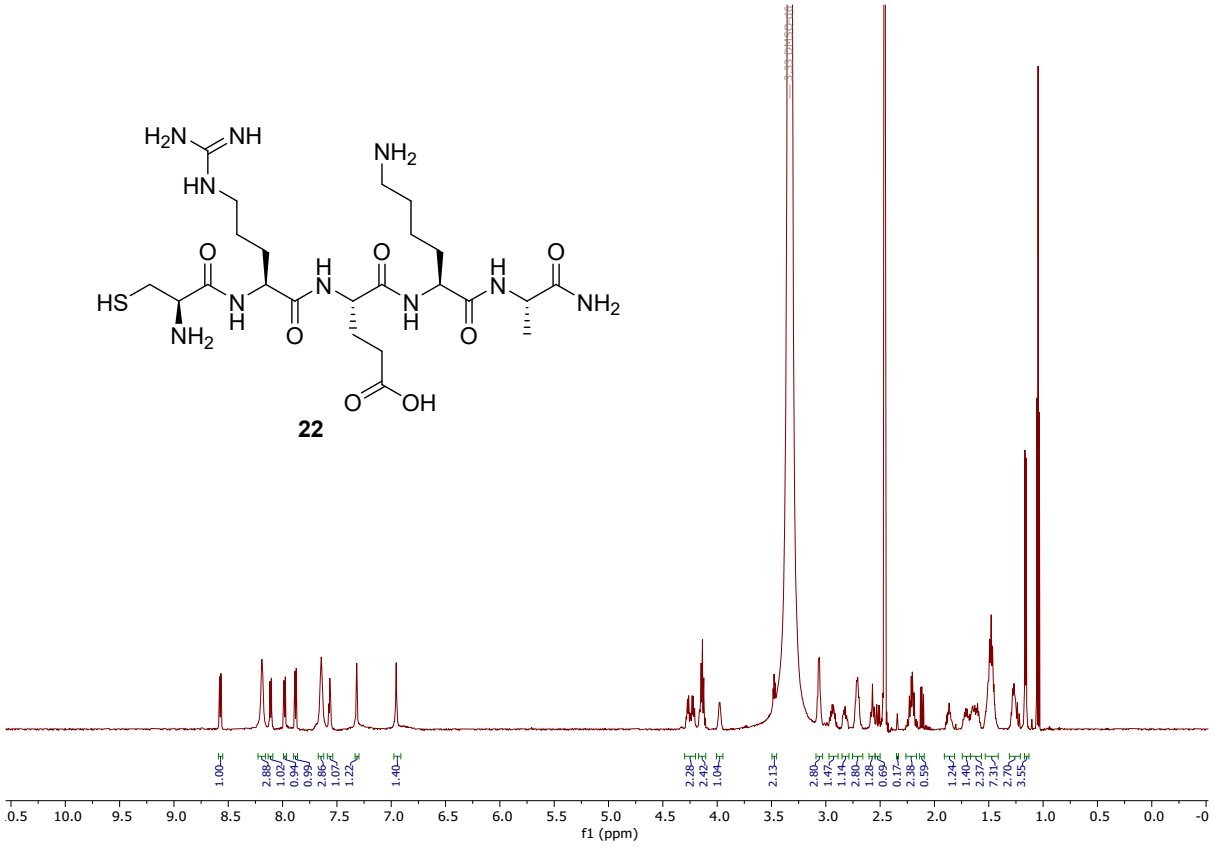
^{1}H and ^{13}C NMR spectra of compound 19



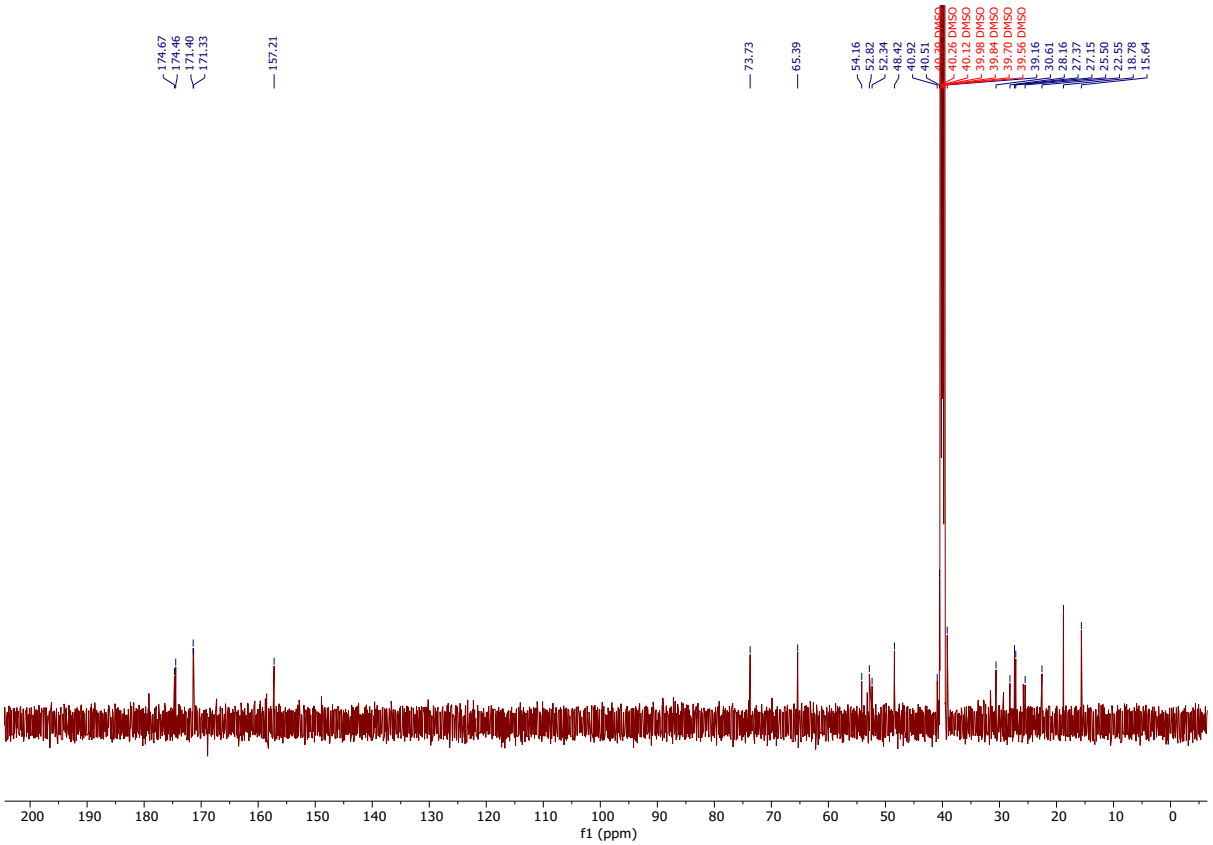
20



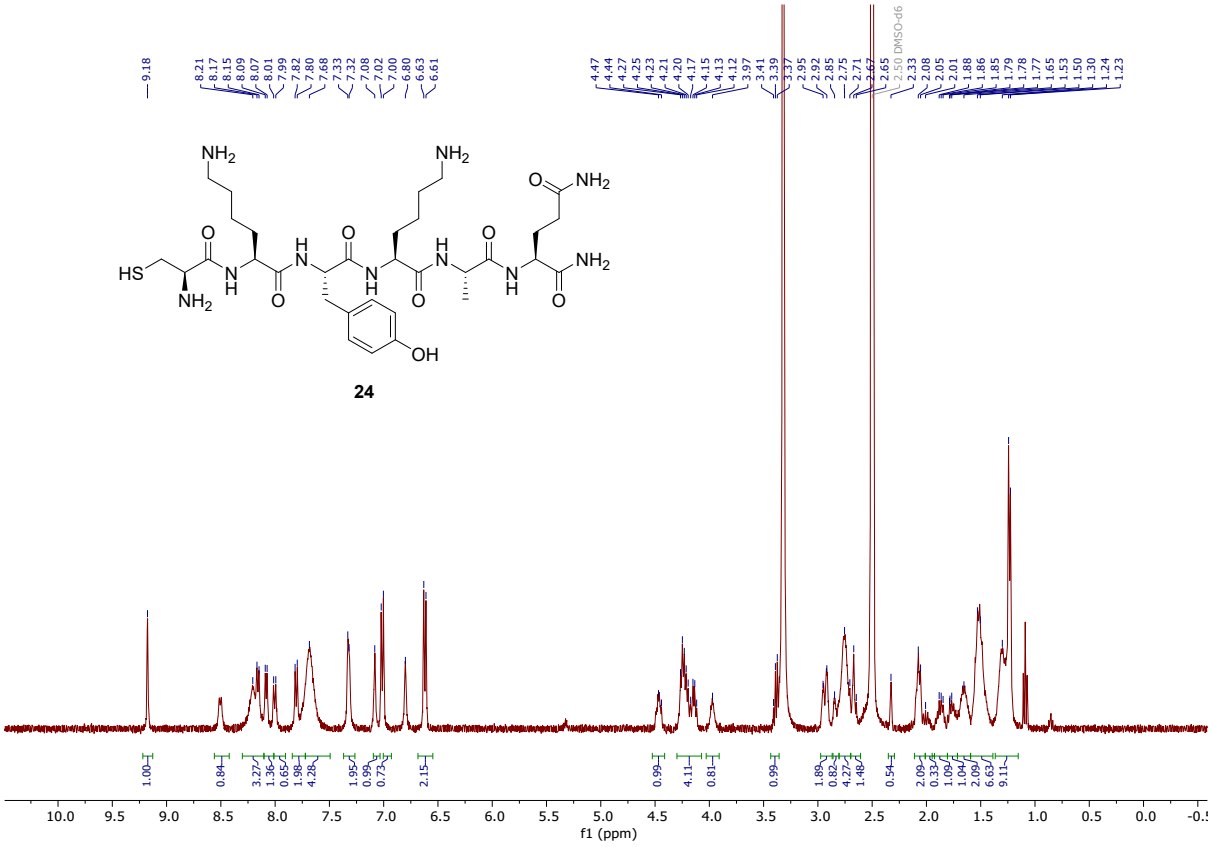
^{1}H and ^{13}C NMR spectra of compound 20



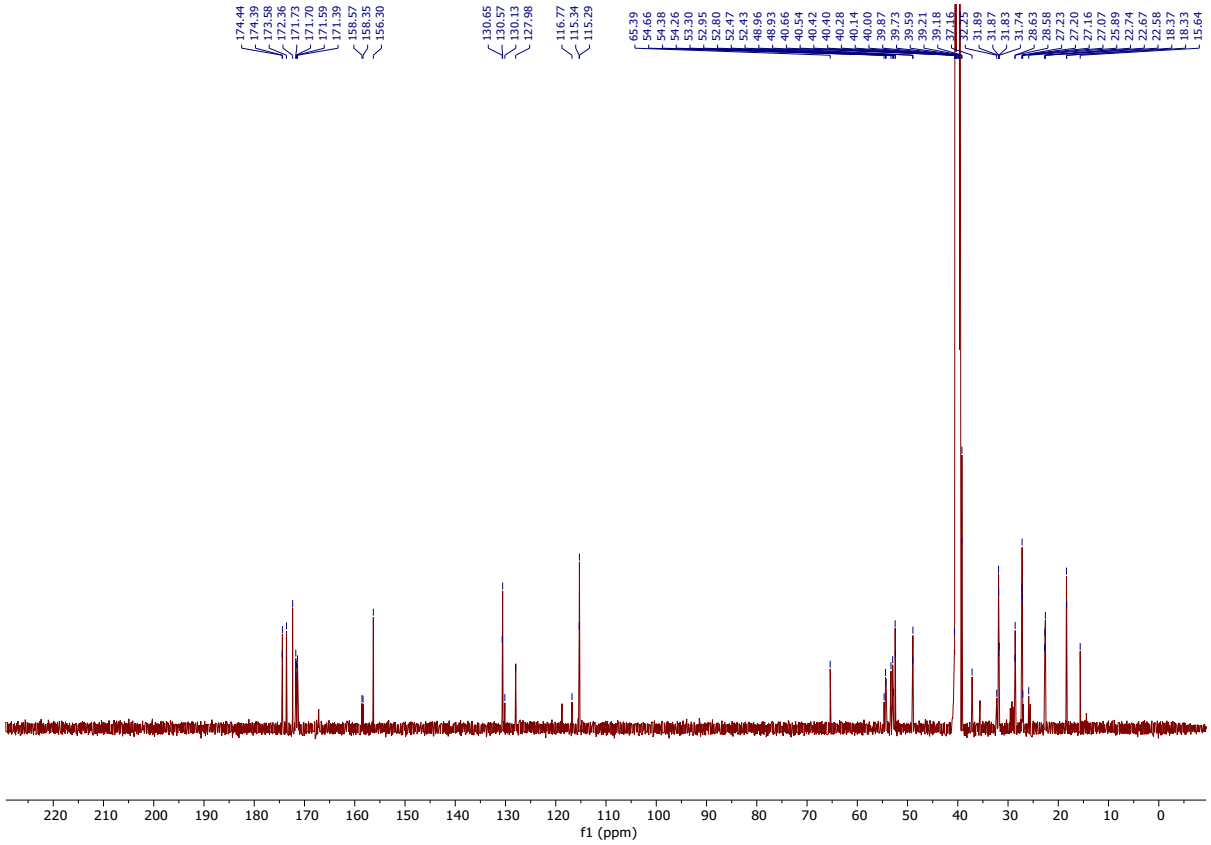
22



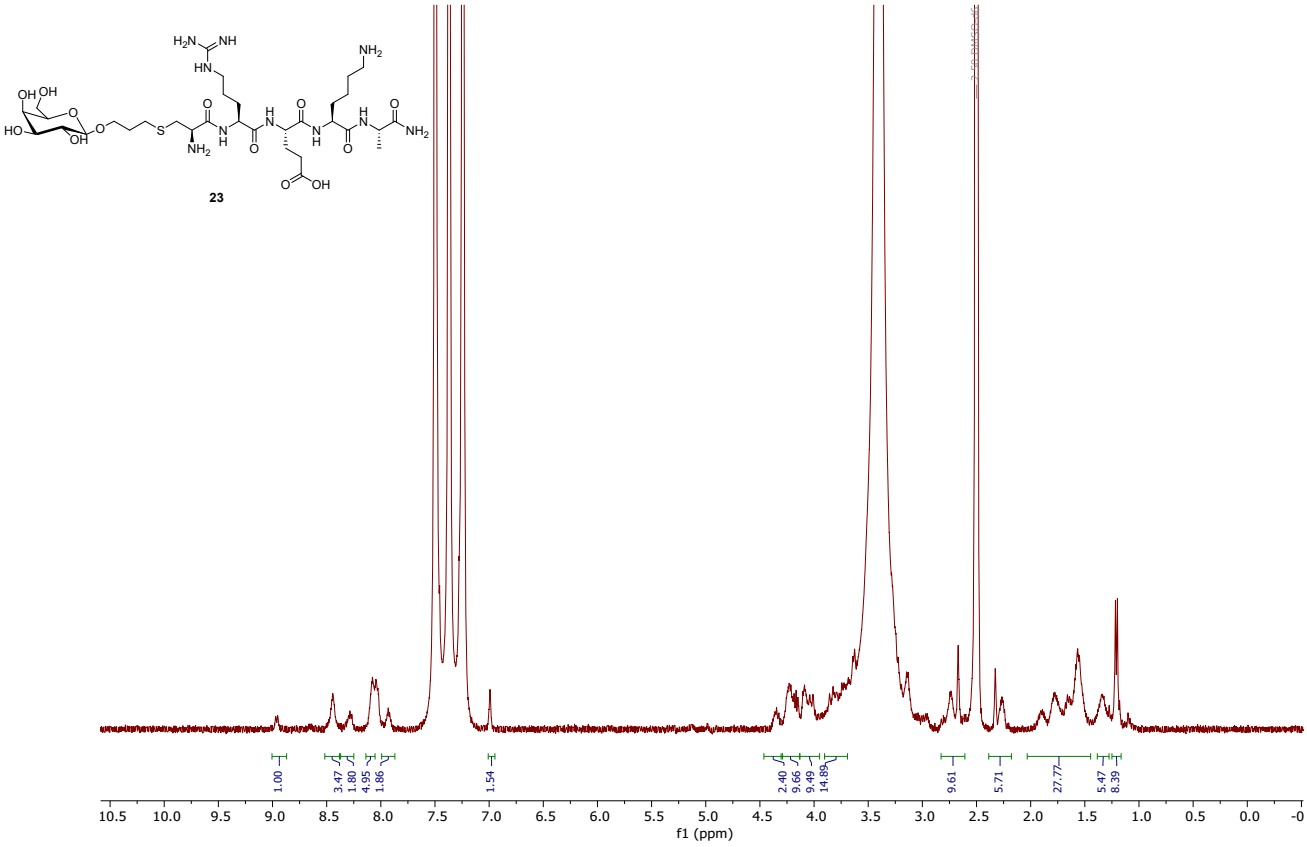
^{1}H and ^{13}C NMR spectra of compound 22

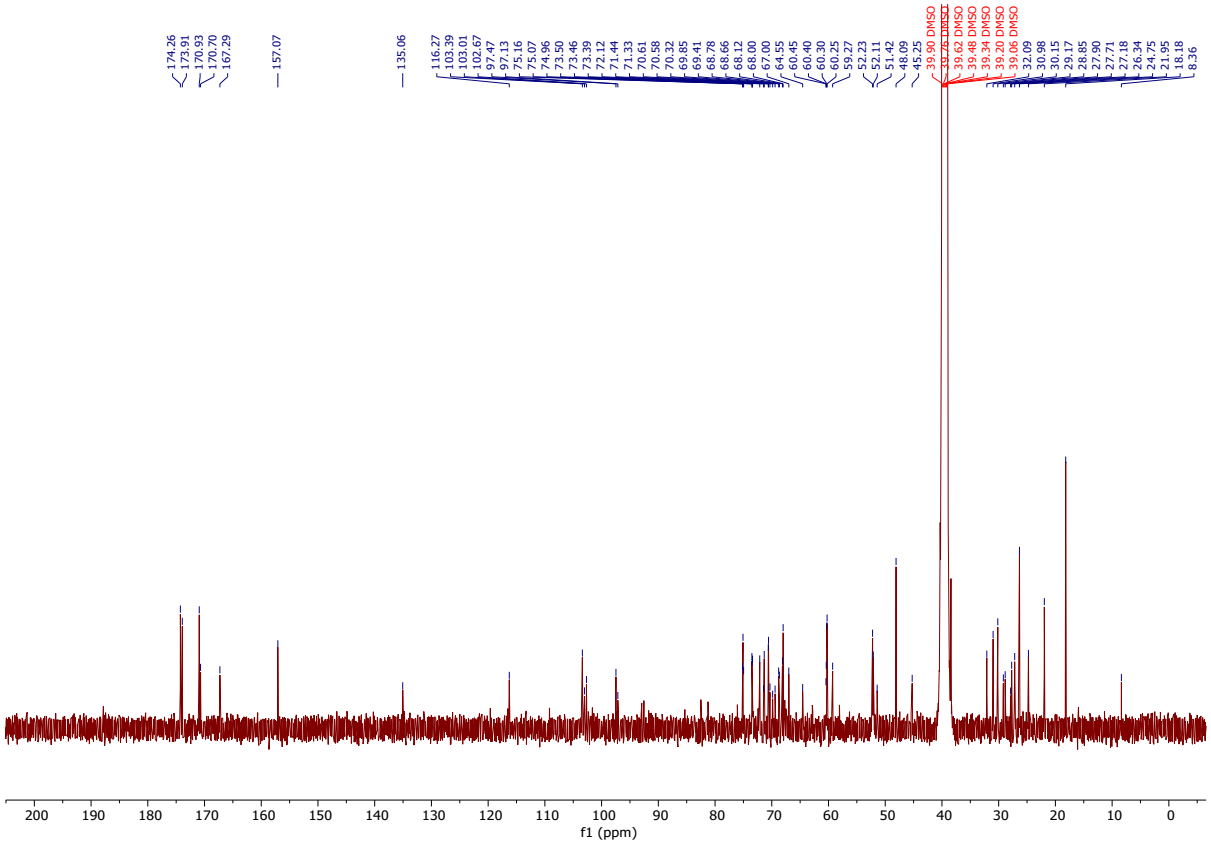


24

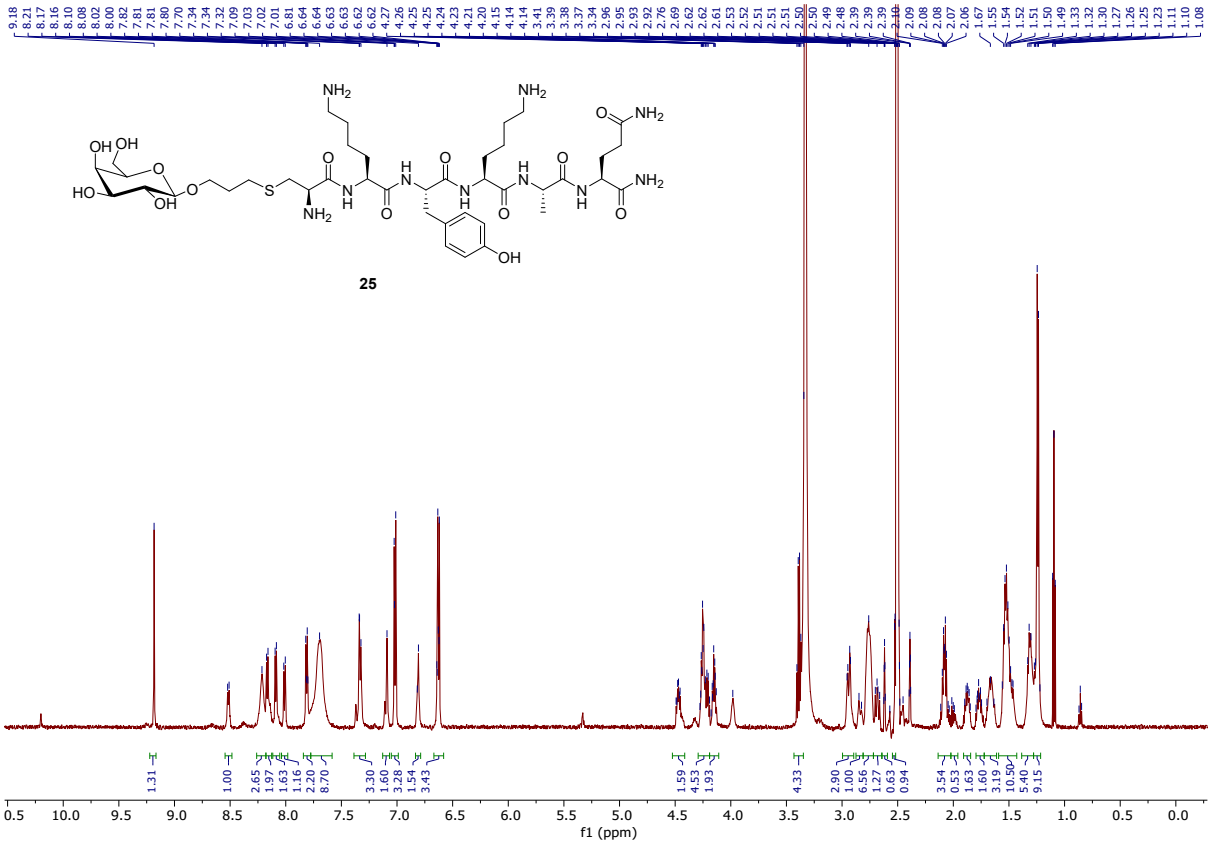


^{1}H and ^{13}C NMR spectra of compound 24

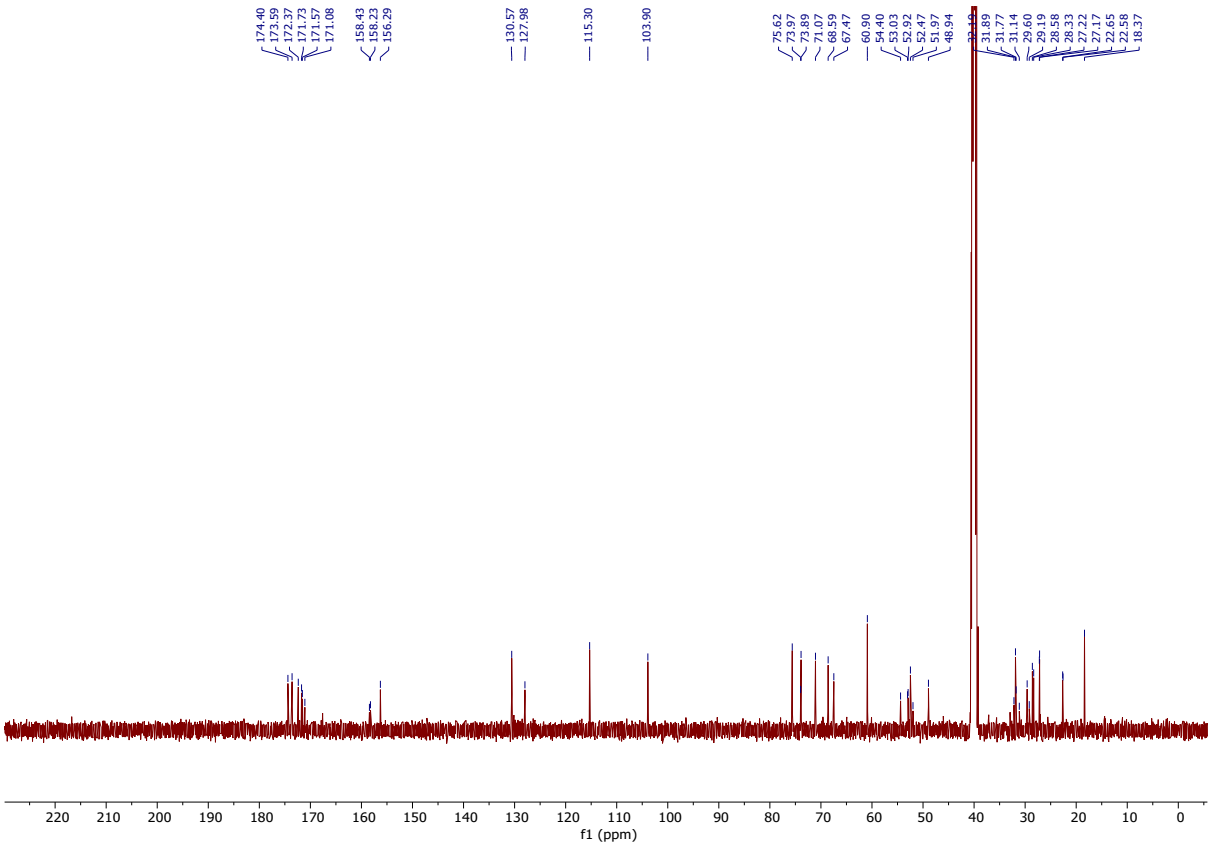




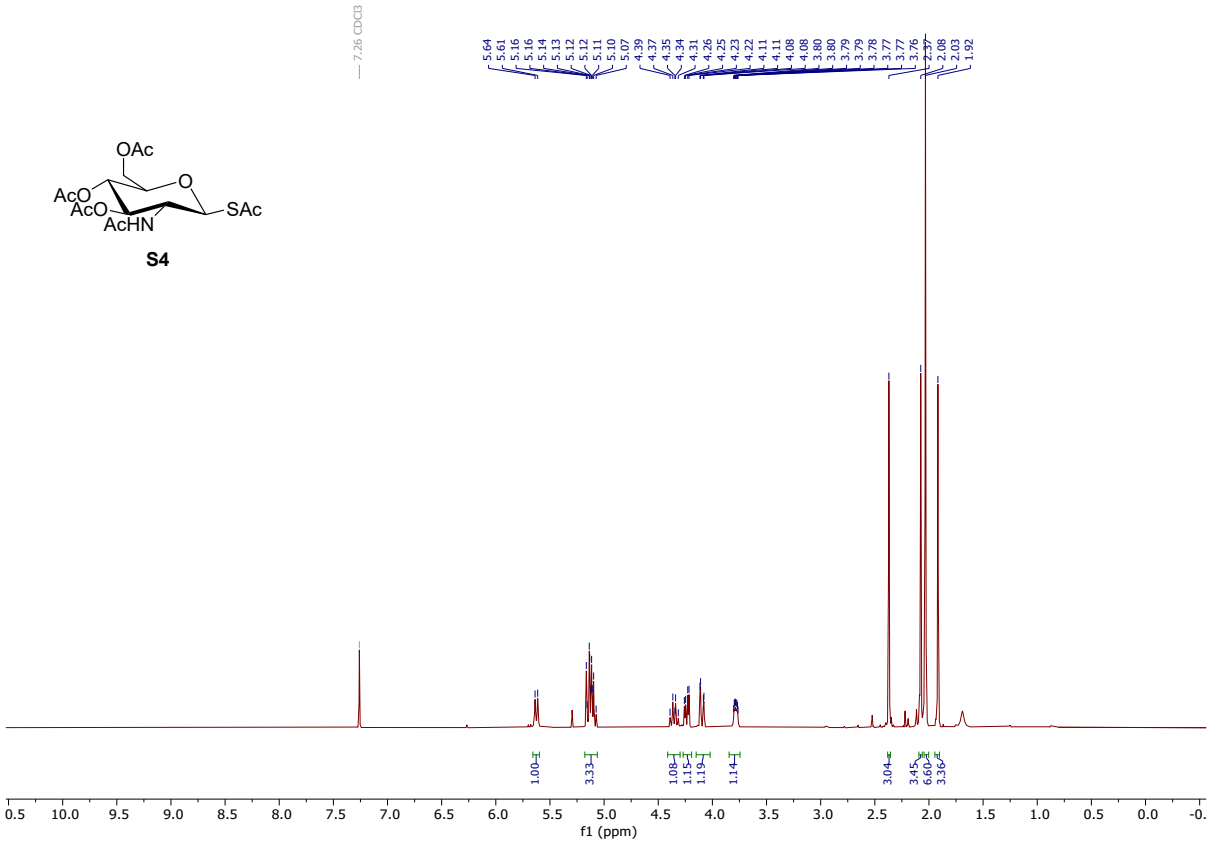
^{1}H and ^{13}C NMR spectra of compound 23

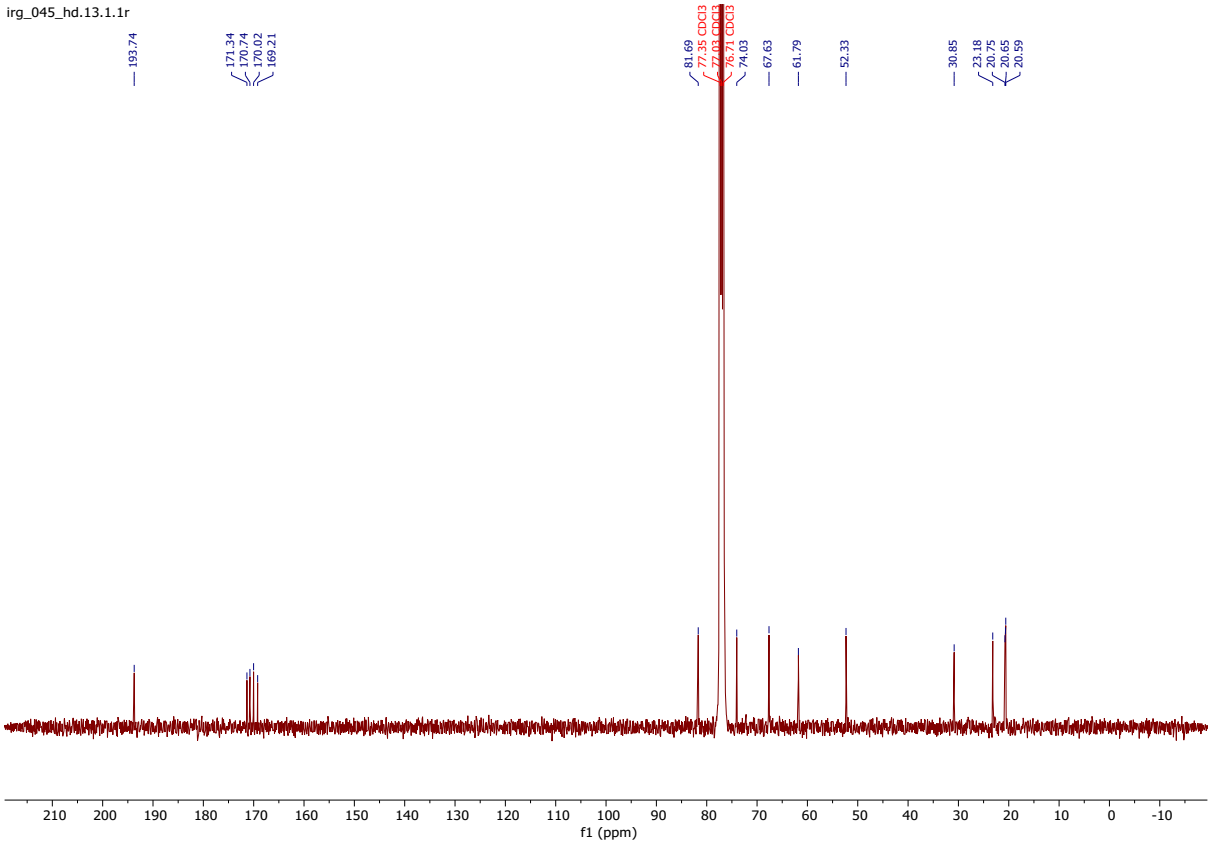


25

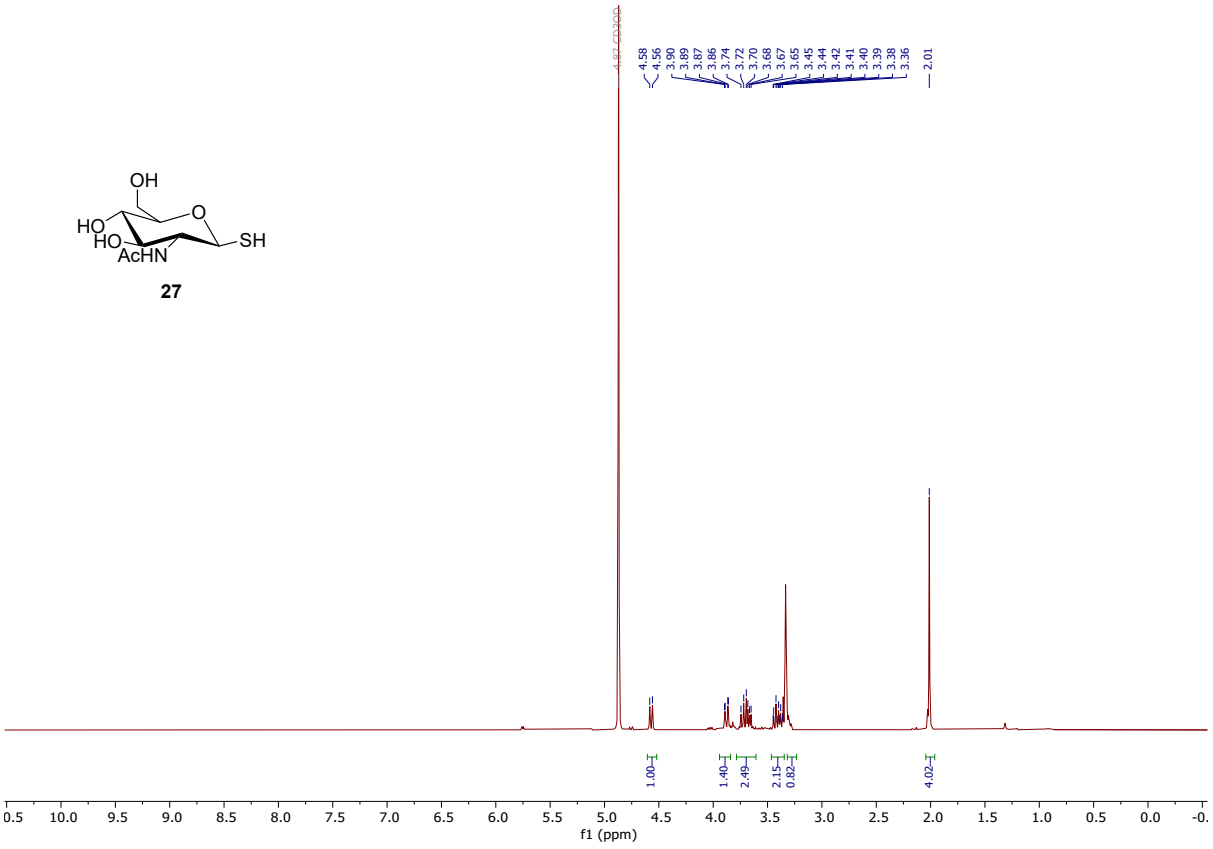


^{1}H and ^{13}C NMR spectra of compound 25

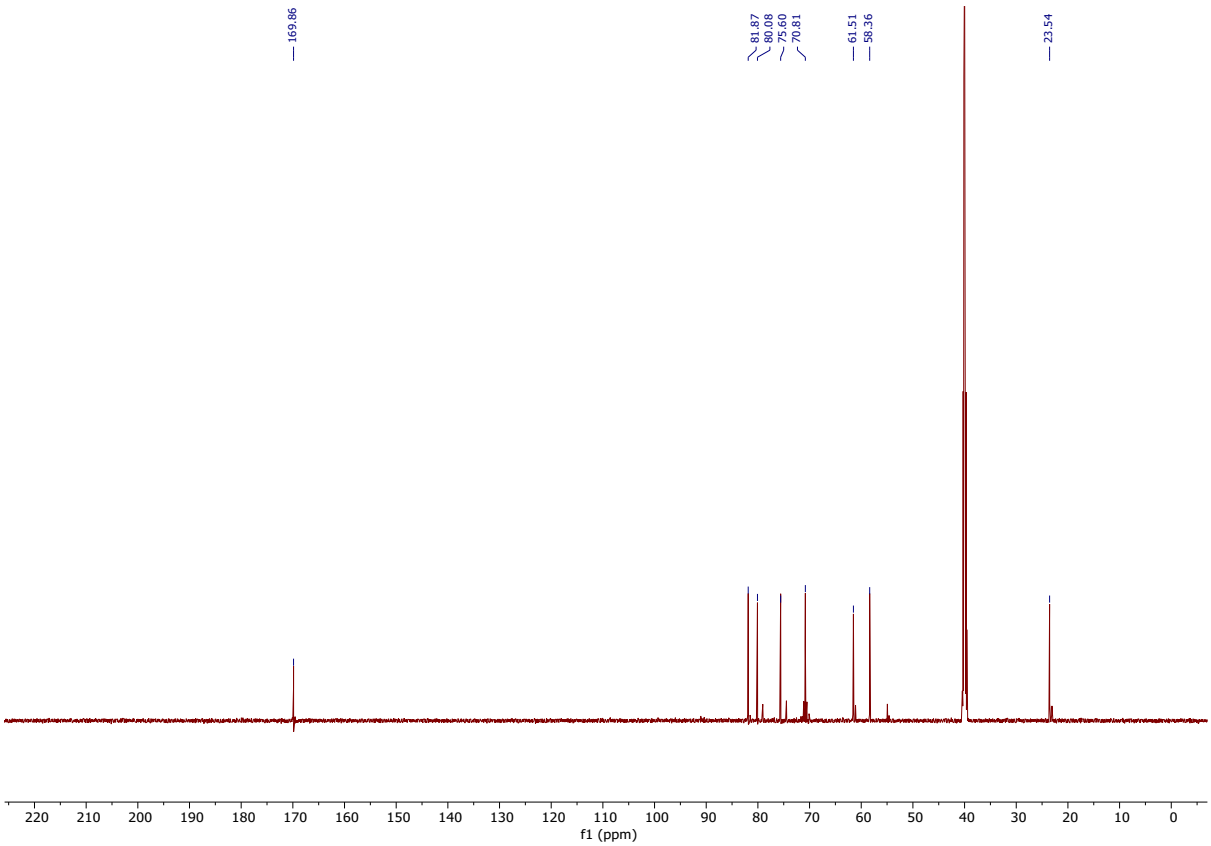




^{1}H and ^{13}C NMR spectra of compound S4.



27



^{1}H and ^{13}C NMR spectra of compound 27.

References

1. B. J. J. Timmer, O. Ramström, *Chem. Eur. J*., 2019, **25**, 14408-14413.
2. M. R. Martinez-Gonzalez, A. Urías-Benavides, E. Alvarado-Martínez, J. C. Lopez, A. M. Gómez, M. Rio, I. Garcia, A. Costela, J. Bañuelos, T. Arbeloa, I. Lopez-Arbeloa, E. Peña-Cabrera, *Eur. J. Org. Chem*., 2014, **91**, *7*, 5659-5663.
3. E.A.Talley, M. D. Vale and E.Yanovsky, *J. Am. Chem. Soc*., 1945, **67**, 2037-2039.
4. D. Hudson, M. H. Lyttle, Eur. Pat. Appl., EP0518295A2, 1992.
5. Afzali-Ardakani, A.; Rapoport, H., *J. Org. Chem.* 1980, **45**, *24*, 4817-4820.
6. S. R. Alexander, D. Lim, Z. Amso, M. A. Brimble, and A. J. Fairbanks, *Org. Biomol. Chem.*, 2017, **15**, 2152–2156.
7. J. Healy, T. Rasmussen, S. Miller, I. R. Booth, S. J. Conway, *Org. Chem. Front*., 2016, **3**, 439-446.